THE SUGARY GENE IN MAIZE AS A MODIFIER OF THE WAXY RATIO[1]

R. A. BRINK

University of Wisconsin, Madison, Wisconsin

Received March 3, 1927

TABLE OF CONTENTS

	PAGE
INTRODUCTION	461
Ratio of waxy grains in pollen from sugary and non-sugary plants segregating for waxy	463
Proportion of waxy seeds in the upper and lower halves of the ear	464
The waxy ratio following the pollination of ears with long and short silks	466
Experiments with pollen mixtures	469
The chemical composition of pollen from sugary and non-sugary plants	476
Comparative weights of $S_u W_x$, $S_u w_x$, $s_u W_x$ and $s_u w_x$ seeds borne on the same ears	478
Rate of growth of sugary and non-sugary sprouts and digestion of the endosperm reserves during seedling development	480
DISCUSSION	484
SUMMARY	490
LITERATURE CITED	491

INTRODUCTION

It has been shown in an earlier paper (BRINK and BURNHAM 1927) that the sugary gene (s_u) in maize may exert a differential action on waxy (w_x) and non-waxy (W_x) male gametophytes leading to marked deficiencies in the waxy ratio. Ordinarily the backcross $w_x w_x$ ♀ × $W_x w_x$ ♂ gives about 48 percent waxy seeds; if the staminate parent is homozygous for the sugary factor, that is, is of the constitution $s_u s_u W_x w_x$, the proportion of seeds in the recessive class is, on the average, about 35 percent. The waxy ratio is modified in this way only when the pollen parent is homozygous for the sugary factor; the double heterozygote, $S_u s_u W_x w_x$, used as the male in combination with $s_u s_u w_x w_x$ plants gives the same waxy ratio in both non-sugary and sugary classes as is obtained following $s_u s_u w_x w_x$ ♀ × $S_u S_u W_x w_x$ ♂ crosses, namely, about 48 percent. This shows that $s_u W_x$ and $s_u w_x$ pollen grains behave differently according to the type of sporophyte which produces them. The sugary gene in the pistillate parent does not alter the ratio. These relationships are illustrated in figure 1.

[1] Papers from the Department of Genetics, Agricultural Experiment Station, UNIVERSITY OF WISCONSIN, No. 75. Published with the approval of the Director of the Station.

The author desires to acknowledge the assistance rendered in this investigation through a grant from the Research Fund of the UNIVERSITY OF WISCONSIN.

To account for this effect of the sugary gene on the waxy ratio the hypothesis was advanced that the cytoplasm contributed by the sporophyte to the microspores is different in sugary and non-sugary plants. It is assumed that waxy male gametophytes receiving sugary-type cytoplasm are handicapped in development to a greater extent than the alternative non-waxy class.

The immediate cause of the deficiency of waxy seeds is believed to be a differential rate of growth in the style of the two classes of male gameto-

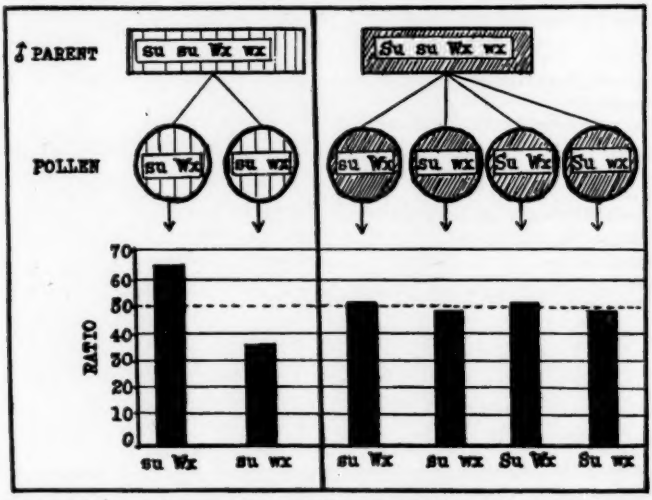


FIGURE 1.—Summary diagram of the modifying effect of the sugary gene on the waxy ratio The light and the heavy crosshatching represent the two respective types of cytoplasm, formed in sugary and non-sugary plants, with which waxy and non-waxy pollen nuclei (small rectangles) are assumed to react differently. The solid rectangles below indicate the proportions of non-waxy and waxy seed resulting when pollen from $s_u\ s_u\ W_x\ w_x$ and $S_u\ s_u\ W_x\ w_x$ individuals, respectively, is applied to $s_u\ s_u\ w_x\ w_x$ plants.

phytes, $s_u\ W_x$ and $s_u\ w_x$, arising from sugary homozygotes during the stage in which the pollen reserves are being utilized; it is assumed that in their later development the two classes of pollen tubes advance toward the ovary at the same rate.

In the present communication we shall present the evidence upon which our conclusion was based regarding the stage at which the selective action occurs. A previous experiment on the effect on the waxy ratio of pollinating long and short silks has been repeated and the results demand a modification of our former deduction in this regard. We have sought also

to determine, by means of pollen mixture experiments, whether $s_u\ W_x$ as well as $s_u\ w_x$ pollen tubes produced by $s_u\ s_u\ W_x\ w_x$ plants are affected by the sugary gene.

Thus far we have been unable to devise a means of directly testing our hypothesis that the sugary gene exerts its differential action through modification of the cytoplasm of the sporophyte nor has it been possible to study the physiological differences in the four classes of pollen involved. Maize pollen can not be satisfactorily cultivated on artificial media and critical observations on early development of the tubes on the silk are attended with difficulty. It appeared, however, that a study of the action of the sugary and the waxy genes in the seed might afford an indirect means of approach to certain aspects of the problem. In the seed, as in the pollen, reserves are laid down and later utilized in vegetative development. It is not improbable that in the two parts these changes involve certain common physiological processes. How far the parallel extends, of course, is a matter of conjecture only. We have no *a priori* assurance that the rate of sprout development in the seedling is limited by the same factors as control elongation of the pollen tube. However, the sugary and the waxy genes occasion striking effects in the seed in regard to the amount and kind of reserves formed and it was thought that a further knowledge of the relationships obtaining here might be helpful in understanding the effects in the pollen. Accordingly we have determined the relative amount of reserves laid down in the endosperms of comparable samples of $S_u\ W_x$, $S_u\ w_x$, $s_u\ W_x$ and $s_u\ w_x$ seeds and have measured the rates at which the respective classes of sprouts develop as the food materials are consumed.

RATIO OF WAXY GRAINS IN POLLEN FROM SUGARY AND NON-SUGARY PLANTS SEGREGATING FOR WAXY

The marked deficiency of waxy seeds following the use of pollen from $s_u\ s_u\ W_x\ w_x$ plants is not due to a disturbance in the ratio in which waxy and non-waxy microspores are formed. Since the waxy character is expressed in the pollen this point can be demonstrated by direct means by mounting the grains in an iodine-potassium iodide solution which stains the waxy class yellow to brownish and the non-waxy group black. Counts obtained on pollen from waxy heterozygotes, homozygous for the non-sugary and sugary genes, respectively, given in table 1, show that in both cases the deviations from expectation on the Mendelian basis are well within the limits of error due to random sampling. The defect in the proportion of waxy seeds on segregating ears cannot be referred, there-

fore, to irregularities at microsporogenesis in $s_u\, s_u\, W_x\, w_x$ plants; the differential action is manifested later in the history of the gametophyte.

TABLE 1

Proportion of waxy grains in the pollen of $S_uS_uW_xw_x$ and $s_us_uW_xw_x$ plants.

CLASS OF PLANT	DISTRIBUTION OF POLLEN GRAINS			PERCENT WAXY GRAINS	DEVIATION FROM 1:1 RATIO IN PERCENT
	Non-waxy	Waxy	Total		
$S_uS_uW_xw_x$	1066	1050	2116	49.62	-0.38 ± 0.74
$s_us_uW_xw_x$	1177	1226	2403	51.02	$+1.02\pm0.69$

PROPORTION OF WAXY SEEDS IN THE UPPER AND LOWER HALVES OF THE EAR

A deficiency in the ratio of waxy seeds such as we have found might well arise as a result of differential development of waxy and non-waxy pollen tubes in the style. A lower rate of growth of the waxy gametophytes as compared with the alternative class would lead to the participation in fertilization of fewer waxy sperm than is called for by Mendelian theory.

If there were a *constant* difference in rate of growth of waxy and non-waxy pollen tubes throughout the entire period of their development we would expect different proportions of waxy seeds at different levels on the ear. If the two classes of tubes elongate at different rates such a gradient should result from the varying distances which the competing tubes must traverse in order to reach ovaries in different positions on the ear.

An experiment was carried out in 1926 with a view of determining whether waxy and non-waxy pollen tubes arising from homozygous sugary plants led to such a graded distribution of the two classes of seeds. The pollen used was gathered from three progenies, R87a, R88c, and R100c, all of which were grown from the non-waxy seed on a single ear. This ear resulted from self-pollination of a $s_u\, s_u\, W_x\, w_x$ plant, R24b-5, and bore 322 seeds of which 8.39 ± 1.63 percent were waxy. This proportion of waxy seeds is, of course, far below expectation on the Mendelian basis. We should expect a marked difference in the ratio of waxy seeds on the upper and lower portions of the ear when pollen from such a stock is used if the deviation is due to a uniformly lower rate of growth of the w_x pollen tubes. The homozygous non-waxy plants in progenies R87a, R88c, and R100c were separated from the heterozygotes in the field during the flowering period according to the reaction of the pollen reserves to iodine; pollen from the latter class only was used in the test.

The distributions of waxy and non-waxy seeds on the upper and lower halves of the 20 ears obtained were recorded separately. The results are given in table 2. Since the pistillate stocks were homozygous for the waxy gene and the pollen parents heterozygous for this factor the proportion of waxy seeds expected on the Mendelian basis is 50 percent. The number actually obtained, however, was only 20.25 percent. The question with which we are concerned at present is whether the condition which leads to this very marked deficiency in the waxy ratio results in a different proportion of the two classes of seeds at different levels on the ear. Summarizing the data we find 19.43 percent waxy seeds in the upper halves of the ears and 21.12 percent in the lower portions. In the last column of table 2 the differences in the waxy ratios in the two parts of the 20 individual ears are given. Applying STUDENT's method of analysis we find

TABLE 2

Distribution of waxy and non-waxy seeds in the upper and lower halves of ears resulting from the combination $S_u S_u w_z w_z \, ♀ \times s_u s_u W_z w_z \, ♂$.

PEDIGREE NUMBER	UPPER PORTION			LOWER PORTION			PERCENT WAXY		DIFFERENCE
	Non-waxy	Waxy	Total	Non-waxy	Waxy	Total	Upper	Lower	Lower-Upper
R87b-20×R87a	126	45	171	100	54	154	26.31	35.06	+ 8.75
-26× "	92	40	132	122	38	160	30.30	23.75	− 6.55
-28× "	97	69	166	131	33	164	41.56	20.12	−21.44
R89b-39×R88c	194	41	235	117	52	169	17.44	30.76	+13.32
-50× "	202	60	262	202	38	240	22.90	15.83	− 7.07
-52× "	153	55	208	145	40	185	26.44	21.62	− 4.82
-55× "	154	41	195	162	37	199	21.02	17.70	− 3.32
-64× "	166	25	191	104	27	131	13.08	20.61	+ 7.53
-65× "	172	35	207	160	51	211	16.90	24.17	+ 7.27
-67× "	108	38	146	111	37	148	26.02	25.00	− 1.02
-74× "	133	19	152	94	31	125	12.50	24.80	+12.30
R90b-36×100c	100	31	131	101	35	136	23.66	25.73	+ 2.07
-37× "	128	43	171	190	76	266	25.14	28.57	+ 3.43
-76× "	209	32	241	194	34	228	13.27	14.91	+ 1.64
-81× "	235	37	272	137	32	169	13.60	18.93	+ 5.33
-83× "	215	30	245	253	36	289	12.24	12.45	+ 0.21
-84× "	235	37	272	201	34	235	13.60	14.46	+ 0.86
-89× "	191	28	219	158	27	185	12.78	14.59	+ 1.81
-80×R88c	155	25	180	164	41	205	13.88	20.00	+ 6.12
-91× "	86	29	115	56	24	80	25.21	30.00	+ 4.79
Totals	3151	760	3911	2902	777	3679			

$$z = \frac{+1.560}{7.630} = 0.204 \qquad \text{Odds} = \text{ca. } 4:1$$

that the average difference is +1.560 percent; the lower portion shows the higher ratio of waxy seeds. The standard deviation of these differences is 7.630 percent giving z the value of 0.204. The corresponding odds that the difference is a significant one are about 4 to 1. In other words, the difference might well be due to chance.

The fact that a significantly greater deficiency of waxy seeds in the lower halves of the ears does not exist eliminates any explanation of the departure from Mendelian proportions on the basis of a *constant* differential rate of growth of the two classes of pollen tubes.

THE WAXY RATIO FOLLOWING THE POLLINATION OF EARS WITH LONG AND SHORT SILKS

During the 1926 season an attempt was made to repeat an experiment earlier carried out (BRINK 1925) concerning the effect on the waxy ratio of pollinating long and short silks. Special precautions were observed to minimize various possible sources of error and to make the accessory conditions in the long and short series closely comparable. The bags used to protect the silks were reinforced at the seams with paper strips attached with waterproof paste; the silks in the two series were of the same age, short-styled ears being obtained by trimming off silks which had attained

TABLE 3

Distribution of waxy and non-waxy seeds in the short-styled series of ears resulting from the application of pollen from $S_uS_uW_xw_x$ plants.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY
	Non-waxy	Waxy	Total	
R86b-10×R88a	261	264	525	50.28
R88b-14×	316	309	625	49.44
-17×	133	124	257	48.25
-28×	229	242	471	51.38
-35×	194	146	340	42.94
-76×	257	259	516	50.19
-79×	199	244	443	55.08
-84×	346	279	625	44.64
-86×	235	347	582	59.62
-87×	215	292	507	57.59
-94a×	281	283	564	50.18
-100.5×	294	272	566	48.06
-102×	80	113	193	58.55
R90b-32×	230	166	396	41.92
-38×	124	111	235	47.23
Totals	3394	3451	6845	50.42±0.41

the same length as those in the long group; the silks were prepared for pollination early in the day (6:00 to 7:30 A.M.) before pollen was flying in the air. In the long series the silks were wrapped lightly with paper and the pollen was applied at the tips. In the short-silked group the average distance from the point of pollination to the tip of the ear was about 2 inches; the corresponding value in the long series was approximately 7 inches. The pollen used each day consisted of a composite lot from several plants; this same mixture was applied to plants in both series.

TABLE 4

Distribution of waxy and non-waxy seeds on the long-styled series of ears resulting from the application of pollen from $S_u S_u W_x w_x$ plants.

PEDIGREE NUMBER	NUMBER OF SEEDS			PERCENT WAXY
	Non-waxy	Waxy	Total	
R86b-14×R88a	40	76	116	65.52
R86c- 8×	32	34	66	51.52
R88b- 4×	101	135	236	57.20
-65×	107	127	234	54.27
-69×	147	185	332	55.72
-70×	79	120	199	60.30
-70×	33	65	98	66.33
-80×	74	97	171	56.72
-82×	98	106	204	51.96
-92×	49	76	125	60.80
-93×	37	53	90	58.89
R90b- 1×	102	100	202	49.50
-26×	23	22	45	48.89
Totals	922	1196	2118	56.47±0.74

Two separate experiments were carried out; in the first, pollen from homozygous sugary plants heterozygous for the waxy factor ($s_u\ s_u\ W_x\ w_x$) was used and in the second, the pollen came from non-sugary plants ($S_u\ S_u\ W_x\ w_x$). The experiment in which sugary pollen was used, failed; 37 ears were obtained in the short-silked group but only 3 small ears bearing 545 seeds were secured from the long-silked plants. The number of ears in the latter group is too small to permit of a valid comparison of the waxy ratios.

In the test in which pollen from non-sugary plants was employed, 15 ears were obtained in the short series and 13 ears in the long. The progeny used as the source of pollen was raised from the seed on an ear (R21–16) which produced 20.58 percent waxy seeds on being selfed. The detailed results are given in tables 3 and 4. The average percentage of waxy seeds in the short-silked series is 50.42±0.41 and in the long series 56.47±0.74.

The proportion of waxy seeds in the long series exceeds that in the short by 6.05 ± 0.85 percent; the difference is over 7 times its probable error and unquestionably significant.

A check on this result is afforded by comparing the average percentages of waxy seeds in the respective groups of long- and short-silked ears pollinated the same day with the same pollen composite. The data summarized in this way are shown in table 5. In each of the four pairs formed, the waxy ratio is higher in the long-silked series. Using STUDENT'S method of analysis we find the average difference to be -5.74 percent and the standard deviation of the difference is 3.38 percent. This gives a value of z equal to 1.69 which corresponds to odds of 34 to 1 that the difference is a significant one.

TABLE 5

Average percentages of waxy seeds on long- and short-silked ears treated with the same respective pollen composites.

POLLEN COMPOSITE (DATE)	PERCENT WAXY SEEDS		DIFFERENCE
	Long silk	Short silk	
August 13	58.52	50.28	−8.24
21	56.95	56.21	−0.74
24	57.20	47.84	−9.36
25	49.19	44.57	−4.62

$$z = \frac{5.74}{3.38} = 1.69 \qquad \text{Odds} = 32:1$$

This result is a surprising one. The deficiency of waxy seeds usually resulting after such a combination is not in evidence. In the long series there is a significant excess of waxy grains as compared with Mendelian expectation. It is recalled that a previous experiment of this type (BRINK 1925) gave significantly fewer waxy seeds in the long-silked series. The difference in the waxy ratio in the long- and short-silked groups cannot be attributed to the operation of accessory factors since samples of the same respective pollen composites were applied to both groups of pistillate plants.

In view of the random distribution of waxy seeds on the ear, discussed earlier in the present paper, indicating that a *constant* difference in rate of growth of waxy and non-waxy pollen tubes does not occur, a significant difference in the waxy ratio following pollination of long and short silks is not expected. Why the results in the long- and short-silked series are not consistent with the other finding, we cannot say; it would appear, however, that determination of the waxy ratio in the upper and lower parts of the ear is the more critical test.

EXPERIMENTS WITH POLLEN MIXTURES

Previous studies with pollen from $s_u\ s_u\ W_z\ w_z$ plants show clearly that the sugary gene in homozygous condition increases the disparity between the observed waxy ratio and the proportion expected on Mendelian grounds. The waxy ratio might be altered by an increase in the effectiveness of $s_u\ W_z$ gametophytes, by a decrease in the ability of $s_u\ w_z$ gametophytes to develop, or by changes in the developmental capacity of both classes as compared with non-sugary pollen tubes. Previous tests do not permit us to make a decision between these three possibilities.

By means of pollen mixtures information on this phase of the problem may be obtained. In 1926 four experiments were carried out with mixtures of pollen from plants of the types $S_u\ S_u\ W_z\ w_z$ and $s_u\ s_u\ W_z\ w_z$. In two of the tests equal weights of the two classes of pollen were mixed and in the remaining two the proportions were 60 percent sugary pollen and 40 percent non-sugary. The pollen mixtures were applied to double recessive individuals ($s_u\ s_u\ w_z\ w_z$). Two progenies were used as pistillate parents; R95a was grown from the $s_u\ w_z$ seed on an ear resulting from a cross of the type $S_u\ s_u\ W_z\ w_z$ ♀ × $s_u\ s_u\ W_z\ w_z$ ♂ (R22–4 × R22a–24) which gave 22.75 percent waxy grains; R95b resulted from a similar combination (R22–5 × R22a–24) which gave 20.73 percent waxy seeds.

The proportions in which the sugary and non-sugary lots of pollen were mixed was determined by weight, precautions being taken to secure samples of uniform moisture content. Maize pollen varies widely in the amount of water it carries, according to the humidity of the atmosphere. Changes in moisture content occur with considerable rapidity, however, and it was thought that by bringing the two samples of pollen to the laboratory and exposing them in thin layers for about 4 hours, an equilibrium would be reached. This procedure was followed and the pollen applied to the silks directly after weighing and mixing.

The numerical proportion of sugary and non-sugary grains in such mixtures as we have used cannot be directly determined. It seems probable, however, that the ratio is not far from that of the weights of the respective samples which were mixed. We have found after adding 25 percent by weight of $s_u\ w_z$ grains to the pollen from individuals of the constitution $S_u\ s_u\ W_z\ w_z$ that the waxy ratio was increased in proportion within the limits of random sampling; addition of the same amount of $s_u\ W_z$ pollen to a sample from the same class of individual ($S_u\ s_u\ W_z\ w_z$) on the other hand, raised the non-waxy percentage to 57.2 rather than to 60 percent as is expected on the 3:2 basis; the difference was statistically significant. The latter fact might be taken to indicate that the individual pollen grains

from $s_u\ s_u\ W_z\ W_z$ plants are somewhat heavier, on the average, than those from the $S_u\ s_u\ W_z\ w_z$ stock. It would appear, however, that we would not be far wrong in assuming that corresponding amounts of pollen of the different classes, measured by weight, contain equal numbers of grains. If the same differences in amounts of reserve materials obtain in the pollen as in the endosperm, we would expect that sugary pollen grains would be about 13 percent lighter than the non-sugary; waxy pollen grains should be approximately 2 percent lighter than non-waxy grains in both the sugary and non-sugary classes (see below in section on comparative weights of seed).

Four pollen mixtures which we have designated, A–50, A–60, B–50, and B–60 were used. The first two of these involve pollen from a sugary stock giving moderately low waxy ratios; B–50 and B–60, on the other hand, represent a line in which the waxy ratio in selfed sugary plants ($s_u\ s_u\ W_z\ w_z$) is only about 11 percent.

The pollen for mixtures A–50 and A–60 was derived from the heterogygous waxy plants in the non-sugary ($S_u\ S_u\ W_z\ w_z$) progeny, R91, and individuals of the composition $s_u\ s_u\ W_z\ w_z$ in progeny R92. The seeds from which these progenies were grown were composite lots taken from sib ears as follows:

	Pedigree number of Parent Ears	*Percent Waxy Seeds*
Progeny R91 (Non-sugary)	R22-25	23.04
	-36	23.61
	-48	20.26
	-49	25.13
	-56	28.29
Progeny R92 (Sugary)	R22a- 9	22.62
	-14	13.12
	-20	19.75

In the mixture A–50 a composite sample of pollen from $S_u\ S_u\ W_z\ w_z$ plants in R91 was combined in equal proportions with a lot gathered from the $s_u\ s_u\ W_z\ w_z$ individuals in R92. As in the other tests to be discussed below, the pollen mixture was applied to $s_u\ s_u\ w_z\ w_z$ plants. The distributions on the resulting ears are given in table 6 and illustrated in figure 2.

The proportion of sugary seeds on this group of ears is 42.08 percent. Assuming that 50 percent of the pollen grains applied were of the sugary type, the ratio of this class of seed is 7.92 ± 0.40 percent in defect of expectation. The difference is unquestionably significant. In the non-sugary group the percentage of waxy seeds is 46.67 ± 0.53, and in the sugary class 37.68 ± 0.62. Both these values are significantly lower than the pro-

portion expected on the Mendelian basis. The difference between the percentage of waxy seeds in the non-sugary and sugary classes amounts to 8.99 ± 0.82; the odds against its being due to chance are extremely high.

It is interesting to recall in this connection the results formerly obtained (BRINK and BURNHAM 1927) when pollen from plants of the composition $S_u\ s_u\ W_x\ w_x$, producing the same four genetic classes of spores as comprise the present mixture, was applied to a double recessive stock. In the

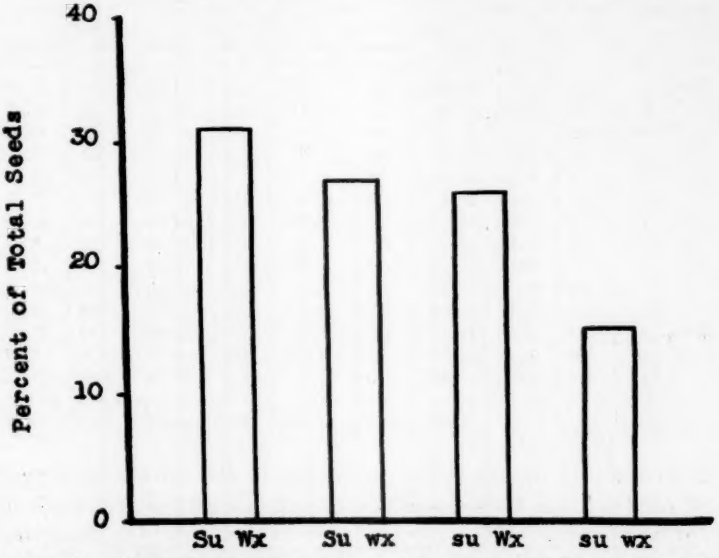


FIGURE 2.—Distribution of the four classes of seeds formed on using pollen mixture A-50 which contained 50 percent by weight of pollen from $S_u\ S_u\ W_x\ w_x$ plants and 50 percent from $s_u\ s_u\ W_x\ w_x$ individuals.

earlier experiment the waxy ratio was not significantly different in the sugary and non-sugary classes. This difference arises from the fact that in the present case the $s_u\ W_x$ and $s_u\ w_x$ pollen grains came from a homozygous sugary sporophyte; in the other test these same types of pollen had their origin in individuals carrying the dominant non-sugary gene. A fact for which the present pollen mixture experiment affords proof is the independence of the respective classes of pollen tubes in the style; non-sugary gametophytes growing in the same silks do not alter the effectiveness of sugary pollen tubes arising from $s_u\ s_u\ W_x\ w_x$ individuals.

The average proportions of the four respective classes of seed on this group of ears show clearly that the $s_u\ w_x$ group is significantly deficient in numbers. The question arises whether the $s_u\ W_x$ class is also low in comparison with the corresponding non-sugary group.

TABLE 6

Distribution of seeds on ears resulting from the application of pollen mixture A-50 (50 $S_uS_uW_xw_x$+ 50 $s_us_uW_xw_x$) to the double recessive, $s_us_uw_xw_x$.

EAR-NUMBER	TOTAL SEEDS	NON-SUGARY				SUGARY			
		Non-waxy		Waxy		Non-waxy		Waxy	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
R95a- 3	557	127	22.80	126	22.62	151	27.11	153	27.47
- 6	569	153	26.89	174	30.58	147	25.83	95	16.69
- 8	590	175	29.66	160	27.12	149	25.25	106	17.97
- 9	573	236	41.19	166	28.97	125	21.81	46	8.03
-10	324	111	34.26	98	30.25	77	23.76	38	11.73
-11	459	135	29.41	124	27.01	118	25.71	82	17.86
-11	602	169	28.07	180	29.90	162	26.91	91	15.12
-12	464	114	24.57	128	27.59	135	29.09	87	18.75
-13	554	172	31.05	166	29.96	149	26.89	67	12.09
-15	486	149	30.66	122	25.10	132	27.16	83	17.08
-17	502	145	28.88	135	26.89	124	24.70	98	19.52
R95b-19	371	116	31.27	106	28.57	100	26.95	49	13.21
-21	529	245	46.31	78	14.74	167	31.57	39	7.37
-28	497	139	27.97	150	30.18	120	24.14	88	17.71
Totals	7077	2186	30.89	1913	27.03	1856	26.22	1122	15.85

If we compare the respective percentages of the two classes of seeds, $S_u\ W_x$ and $s_u\ W_x$ on the series of 14 ears we find in 12 cases the frequency of the latter phenotype is lower and in two cases higher. On the average, the proportion of $s_u\ W_x$ grains is lower by 4.722 percent. We may evaluate this difference by STUDENT'S method; the standard deviation of the difference proves to be 6.244 percent, giving z the value 0.756. The odds, therefore, are about 120 to 1 that the difference is a significant one.

Mixture A–60 was made up from the same composite samples of pollen as entered into A–50, the only difference being that in mixture A–60 the proportion of sugary to non-sugary pollen was 60 to 40 rather than 50 to 50. It will be noted from the summary in table 7 that the percentage of sugary seeds is again below expectation; the observed proportion is 52.23 percent, whereas 60 percent is called for. Again the proportion of waxy seeds is much lower in the sugary than in the non-sugary class, the respective values being 38.27 percent and 48.08 percent.

If no selective action were manifested at any stage following the application of the mixture of pollen, we should expect to find sugary and non-sugary seeds in the non-waxy class ($s_u\ W_x$ and $S_u\ W_x$) present on the ears in the ratio of 3:2. The total number of non-waxy seeds in the population is 3,873; of this number 2,185 are sugary and 1,688 are non-sugary. The values expected on the 3:2 basis are 2,324 and 1,549 respectively. There are 139 ± 20 fewer $s_u\ W_x$ seeds than are called for. Not only is the double recessive class ($s_u\ w_x$) in defect, therefore, but the $s_u\ W_x$ group as well.

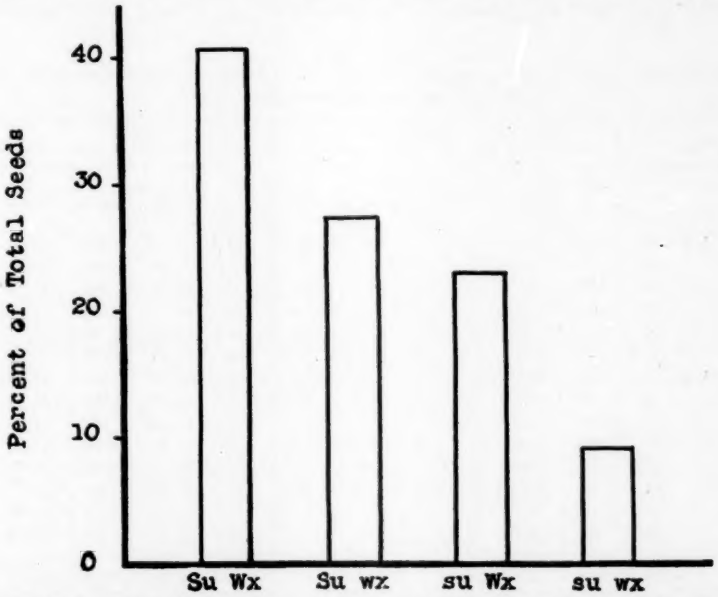


FIGURE 3.—Distribution of the four classes of seed formed in using pollen mixture B-50 containing 50 percent pollen from $S_u\ S_u\ W_x\ w_x$ plants and 50 percent from a closely related low-waxy $s_u\ s_u\ W_x\ w_x$ stock.

As we have noted above, pollen mixtures B–50 and B–60 are representative of a line in which the sugary plants give very low waxy ratios. The non-sugary progeny used, R93, was grown from the non-waxy seeds on two selfed ears, R24a–7 and R24a–8, which bore 23.18 percent and 24.07 percent waxy seeds respectively. The sugary progeny, R94, was raised from seed on three selfed ears obtained in the homozygous sugary progenies ($s_u\ s_u\ W_x\ w_x$) R24b and R24c; these ears contained 13.84 percent, 12.32 per cent, and 7.05 percent waxy seeds. Progenies R24a and

R24c were sibs; R24b consisted of a composite of the same stock from which R24a and and R24c were derived.

Mixture B–50 contained equal parts by weight of $S_u\ S_u\ W_x\ w_x$ pollen from progeny R93 and $s_u\ s_u\ W_x\ w_x$ pollen from R94. The distributions on the ears resulting from its application to $s_u\ s_u\ w_x\ w_x$ plants are given in table 8 and shown graphically in figure 3. Assuming that equal weights represent equal numbers of pollen grains it is expected that one-half the seeds on these ears will be sugary if no selective action intervenes between pollination and fertilization. The proportion of sugary seeds actually

TABLE 7

Distribution of seeds on ears resulting from the application of pollen mixture A-60 (40 $S_u S_u W_x w_x$ + 60 $s_u s_u W_x w_x$) to the double recessive, $s_u s_u w_x w_x$.

EAR-NUMBER	TOTAL SEEDS	NON-SUGARY				SUGARY			
		Non-waxy		Waxy		Non-waxy		Waxy	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
R95a-19	458	110	24.02	90	19.65	153	33.41	105	22.92
-20	557	149	26.75	136	24.42	167	29.98	105	18.85
-23	645	155	24.03	155	24.03	198	30.70	137	21.24
-27	467	118	25.27	116	24.84	148	31.69	85	18.20
-29	370	69	18.65	73	19.73	137	37.03	91	24.57
-30	427	99	23.18	87	20.37	162	37.94	79	18.50
R95b- 1	442	124	28.05	96	21.72	145	32.80	77	17.42
- 2	525	121	23.05	136	25.90	157	29.90	111	21.14
- 9	499	125	25.05	111	22.24	156	31.26	107	21.44
-10	466	104	22.32	103	22.10	166	35.62	93	19.96
-12	545	145	26.60	123	22.57	180	33.03	97	17.80
-13	485	122	25.15	106	21.85	151	31.13	106	21.85
-14	477	103	21.59	115	24.11	148	31.03	111	23.27
-24	442	144	32.58	116	26.24	117	26.47	65	14.70
Totals	6805	1688	24.80	1563	22.97	2185	32.11	1369	20.12

found, however, is only 26.64 percent. The deficiency of 23.36 ± 0.53 percent is clearly significant and indicative of a very pronounced handicap upon sugary male gametophytes. The waxy ratio in the non-sugary group is 39.92 ± 0.63 percent; this is significantly in defect of expectation on the Mendelian basis. In the sugary class the waxy ratio is much lower amounting to 31.12 ± 1.02 percent. The difference between these waxy ratios, 8.80 ± 1.20 percent, is certainly not due to chance.

If non-waxy, sugary pollen tubes ($s_u\ W_x$) are as effective in development as the non-waxy, non-sugary type ($S_u\ W_x$) we should expect equal numbers of $S_u\ W_x$ and $s_u\ W_x$ seeds on these ears. This is far from true, however; the latter class shows a large deficiency. If we apply STUDENT's

method in determining whether the differences in proportion of $S_u\ W_z$ and $s_u\ W_z$ seeds on these ears is significant we find that the average difference is 25.925 percent and the standard deviation of the differences, 3.964; the value of z is 6.54, which gives odds of many thousand to one

TABLE 8

Distribution of seeds on ears resulting from the application of pollen mixture B-50 (50 $S_u S_u W_z w_z$ +50 $s_u s_u W_z w_z$) to the double recessive, $s_u s_u w_z w_z$.

EAR NUMBER	TOTAL SEEDS	NON-SUGARY				SUGARY			
		Non-waxy		Waxy		Non-waxy		Waxy	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
R95a- 2	554	234	42.24	173	31.23	81	14.62	66	11.91
- 4	449	245	54.56	69	15.37	108	24.05	27	6.01
- 5	781	367	46.99	220	28.17	155	19.85	39	4.99
-10	491	183	37.27	145	29.53	97	19.75	66	13.44
R95b- 6	534	228	42.70	163	30.52	104	19.47	39	7.30
-15	545	234	42.93	178	32.66	93	17.06	40	7.34
-16	265	111	41.89	100	37.73	30	11.32	24	9.06
-18	397	168	42.32	128	32.24	69	17.38	32	8.06
Totals	4016	1770	44.07	1176	29.28	737	18.35	333	8.29

that the proportion of $s_u\ W_z$ seeds is actually lower than that of the $S_u\ W_z$ grains. It seems particularly clear in this case that the $s_u\ W_z$ frequency is low as well as that of the $s_u\ w_z$ class of seeds.

TABLE 9

Distribution of seeds on ears resulting from the application of pollen mixture B-60 (40 $S_u S_u W_z w_z$+ 60 $s_u s_u W_z w_z$) to the double recessive, $s_u s_u w_z w_z$.

EAR NUMBER	TOTAL SEEDS	NON-SUGARY				SUGARY			
		Non-waxy		Waxy		Non-waxy		Waxy	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
R95a-10	465	165	35.48	95	20.43	115	24.73	90	19.35
-12	557	265	47.58	146	26.21	104	18.67	42	7.54
-12	532	229	43.04	173	32.52	81	15.22	49	9.21
-16	451	196	43.46	134	29.71	102	22.62	19	4.21
-30	499	193	38.68	152	30.46	119	23.85	35	7.01
R95b-20	487	194	39.83	122	25.05	132	27.10	39	8.01
-27	592	221	37.33	151	25.51	175	29.56	45	7.60
-29	440	159	36.14	126	28.64	104	23.64	51	11.59
Totals	4023	1622	40.32	1099	27.32	932	23.17	370	9.20

In table 9 the results are shown of the experiment with pollen mixture B–60 which consisted of 40 percent pollen from $S_u\ S_u\ W_z\ w_z$ plants and 60

percent pollen from a $s_u\ s_u\ W_z\ w_z$ progeny. It will be seen that increasing the proportion of pollen from $s_u\ s_u\ W_z\ w_z$ plants to 60 percent has raised the frequency of $s_u\ W_z$ seeds to only 23.17 percent. Furthermore, the expected zygotic proportion in the $s_u\ w_z$ class is far from realized.

The results with pollen mixtures B–50 and B–60 definitely show that not only does the sugary gene in homozygous condition so affect the male gametophytes that the $s_u\ w_z$ class of seed is in defect of expectation, but the effective development of $s_u\ W_z$ pollen tubes, as compared with that of the corresponding non-sugary type ($S_u\ W_z$) is also lowered.

THE CHEMICAL COMPOSITION OF POLLEN FROM SUGARY AND NON-SUGARY PLANTS

It appeared possible that the marked difference in the ability of waxy gametophytes arising from sugary and non-sugary plants to develop might be due to a difference in the amount or kind of reserve materials stored in the pollen. If this were the explanation we should expect a significantly greater difference between the composition of waxy and non-waxy pollen coming from sugary plants than between waxy and non-waxy pollen borne by non-sugary stocks.

Pollen was collected for analysis from four progenies of the composition $S_u\ S_u\ W_z\ W_z$, $S_u\ S_u\ w_z\ w_z$, $s_u\ s_u\ W_z\ W_z$ and $s_u\ s_u\ w_z\ w_z$ respectively. The four stocks traced back through self-pollinated parents to a common selfed grandparent. A number of ears were available of each of these four types and composite samples of seed of each stock were planted.

After collection, the pollen was quickly dried at 40°C and placed in a dessicator over concentrated sulfuric acid. The standard methods approved by the Association of Official Agricultural Chemists were used in analysis of the material.

TABLE 10

Proximate analysis of corn pollen. Values given in percent on the moisture-free basis.

	CLASS OF POLLEN			
	Non-Sugary		Sugary	
	Non-waxy	Waxy	Non-waxy	Waxy
Free reducing sugars	0.63	0.76	0.52	0.88
Sucrose	13.61	15.97	15.54	15.24
Total sugars	14.24	16.73	16.06	16.12
Dextrin	1.14	1.30	1.20	1.25
Starch	15.63	15.58	14.26	14.04
Fats	21.94	25.54	23.58	26.76

The percentage composition with respect to the principal carbohydrates, except cellulose, and the fats are given in table 10. The content of reducing sugars, sucrose and dextrin, are not very different in these four classes of pollen, at least they do not show any relationship which appears significant in the present connection. There is a conspicuous difference,

TABLE 11

Percentages of total fats from pollen which are ether soluble.

	CLASS OF POLLEN			
	Non-sugary		Sugary	
	Non-waxy	Waxy	Non-waxy	Waxy
Total fats	21.94	25.54	23.58	26.76
Percent of total, ether sol.	63.22	64.37	73.06	68.98

however, in regard to starch content in the sugary and non-sugary classes. If the amount of starch in the double dominant group, $S_u\ S_u\ W_x\ W_x$ is taken as 100.0 then the non-sugary waxy, $S_u\ S_u\ w_x\ w_x$, contains 99.7; the corresponding value for the $s_u\ s_u\ W_x\ W_x$ class is 91.2 and that for the double recessive, $s_u\ s_u\ w_x\ w_x$, 89.8.

It is noteworthy that the two sugary classes of pollen contain higher percentages of fats than the corresponding non-sugary samples. Both classes of waxy pollen are higher in fat than the respective non-waxy classes; the difference in the non-sugary pair in this regard is 3.6 percent and in the sugary pollen approximately 3.2 percent.

It would appear, moreover, that there are differences in the character of the fats in sugary and non-sugary pollen. The respective percentages of the total (alcohol-soluble) fats which are soluble in ether are given in table 11. Both $s_u\ W_x$ and $s_u\ w_x$ pollen grains show a higher proportion of ether soluble fats than the corresponding non-sugary classes.

The analytical results demonstrate very clearly that there are differences in chemical composition between sugary and non-sugary pollen. They show, moreover, that within each of these groups the waxy and non-waxy classes differ. It is not possible to state with the evidence at hand just how these differences are related to pollen tube growth but it seems not improbable that male gametophytes showing such variations in composition begin development in the style at somewhat different rates. The results do not indicate, however, a greater difference in composition of waxy and non-waxy pollen in the sugary class than in the non-sugary.

COMPARATIVE WEIGHTS OF $S_u\ W_x$, $S_u\ w_x$, $s_u\ W_x$ AND $s_u\ w_x$ SEEDS BORNE ON THE SAME EARS

To determine the effects of the sugary and the waxy genes on relative amounts of reserves synthesized under comparable conditions the four classes of seeds on a series of ears resulting from crosses of the type $s_u\ s_u\ w_x\ w_x$ ♀ $\times S_u\ s_u\ W_x\ w_x$ ♂ were weighed. It was thought that there might be a parallel between the action of these genes in the synthesis of reserves in the endosperm and their behavior in pollen arising from sugary and non-sugary plants. The point we wished to determine particularly was whether the difference between the weights of waxy and non-waxy seeds in the sugary class was greater than that in the non-sugary group.

Only healthy well-developed ears were used in the experiment. The irregularly shaped grains borne on the butts and tips of the ears were discarded. Individual seed weights were taken on the remainder. In the case of the non-sugary seeds classification as to the waxy or non-waxy condition was made prior to weighing but in the sugary group where the waxy individuals can only be distinguished by chipping and staining with iodine the seeds were classified as they came from the scale pan and the

TABLE 12

Average weights in milligrams of $S_u\ W_x$, $S_u\ w_x$, $s_u\ W_x$ and $s_u w_x$ seeds borne on the same ears.

PEDIGREE NUMBER	SMOOTH			SUGARY		
	Non-waxy	Waxy	Difference	Non-waxy	Waxy	Difference
R99-1×P. C. R96a	175.30	173.25	−1.05	160.34	157.06	−3.28
R99-9×	118.86	114.91	−3.95	104.44	102.02	−2.42
R99-13×	122.38	117.13	−5.25	108.99	109.75	+0.76
R99-14×	167.24	167.04	−0.20	149.47	146.48	−2.99
R99-25×	137.03	134.44	−2.59	123.26	118.76	−4.50
R99-30×	159.39	154.84	−4.55	143.45	142.29	−1.16
R99-86×P. M. B	157.58	152.93	−4.65	133.97	132.41	−1.56
R131-47×R129-18	229.40	232.85	+3.45	206.58	208.39	+1.81
R131-58×R133-2	200.13	200.42	+0.29	191.78	190.24	−1.54
R131- 5×R129-21	220.52	215.19	−5.33	191.60	183.08	−8.52
R131-57×R129-1	239.88	239.05	−0.83	192.86	193.10	+0.24
Average	175.25	172.91	−2.24	155.16	153.05	−2.10

weights recorded as referring to a W_x or a w_x seed. The weights were determined by means of a balance of the type SAX (1921) has described but with a change which permitted of readily adjusting the arm if, for any reason, it is slightly displaced with reference to the graduated scale from which the weights are read. The modification consists simply in substituting a burette stand, with an adjustable clamp to carry the glass

arm, in place of the one-piece rigid upright which SAX describes. With this balance one may rapidly weigh individual corn grains accurately to within 2 milligrams.

The average weights of the four respective classes of seeds on the series of 11 ears are given in table 12. Figure 4 shows graphically the relative weights of the four classes, based on the totals, the $S_u\ W_x$ seeds being taken as 100.

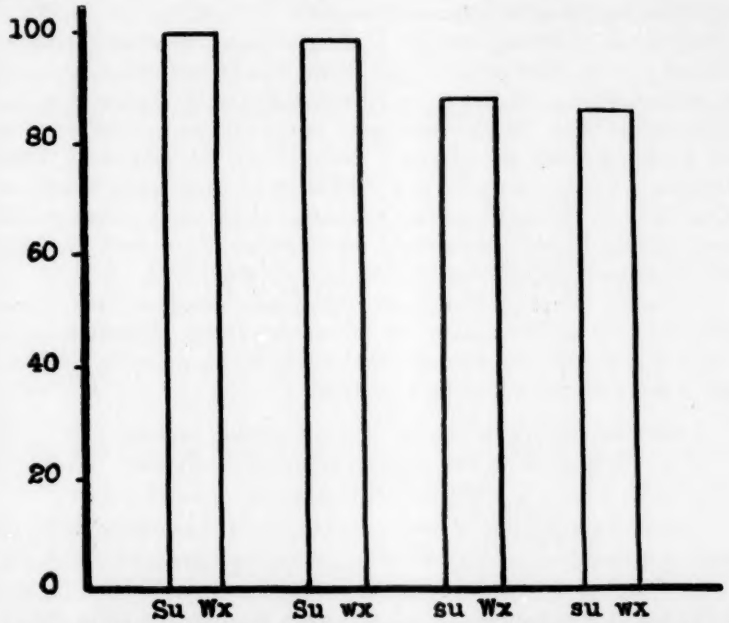


FIGURE 4.—The weight relations of the four classes of seeds on ears resulting from the cross $s_u\ s_u\ w_x\ w_x$ ♀ × $S_u\ s_u\ W_x\ w_x$ ♂. The weight of the $S_u\ W_x$ type is taken as 100.

Relative seed weight may be taken as a fairly close index of the amount of reserves in the endosperm. In other words the differences in weight of these four classes of seeds are largely due to variations in the amount of stored food material. Some further measurements we have made show, however, that the embryo in sugary seeds is actually about 18 percent heavier than that of non-sugary seeds on the same ear; but the embryo comprises only about 12–15 percent of the total weight of the seed. We have not adjusted our figures on this basis and the fact should be borne in mind that the actual difference in weight of sugary and non-sugary endosperms is somewhat greater than the seed weights indicate.

Assuming that the conditions which govern deposition of food materials in the pollen are essentially like those prevailing in the seed we would expect to find, if the difference in the waxy ratio given by $S_u\ S_u\ W_x\ w_x$ and $s_u\ s_u\ W_x\ w_x$ staminate plants is due to a difference in amount of stored material in the pollen, that the weight of $s_u\ w_x$ seeds relative to the weight of $s_u\ W_x$ grains is lower than that of $S_u\ w_x$ seeds compared with the double dominant class, $S_u\ W_x$. In other words, the double recessive class should show a disproportionate decrease in weight.

Analysis of the average weights of the four classes of seeds on the individual ears by STUDENT'S method shows that in both the non-sugary and sugary classes the waxy genotype is significantly lighter than the allelomorphic class. In the non-sugary group the average difference is 2.24 milligrams and the standard deviation of the differences 2.682 milligrams. This gives a value of z of 0.835 which corresponds to odds of about 76:1. In the sugary group the average difference in weight is 2.10 mgms. and the standard deviation of the differences, 2.691 mgs. The odds that the difference is significant in this case are about 57:1.

It turns out, therefore, that the average of the differences in the sugary class is slightly smaller than in the non-sugary group. Clearly there is no evidence of a disproportionate effect of the double recessive combination on the reduction of the food reserves.

RATE OF GROWTH OF SUGARY AND NON-SUGARY SPROUTS AND DIGESTION OF THE ENDOSPERM RESERVES DURING SEEDLING DEVELOPMENT

So far as the utilization of reserves is concerned the young seedling of maize is probably a similar sort of physiological system as the young pollen tube. It was thought, if the deficiency of waxy seeds following the application of pollen from $s_u\ s_u\ W_x\ w_x$ plants was due to a lower rate of development of $s_u\ w_x$ pollen tubes during the period these are dependent upon the initial reserves, that $s_u\ w_x$ seedlings might show a parallel behavior. Accordingly the rate of sprout development in $S_u\ W_x$, $S_u\ w_x$, $s_u\ W_x$ and $s_u\ w_x$ seeds was studied; measurements on the loss in weight of the seed residue occasioned by the consumption of the endosperm reserves, were made at the same time.

Four clean ears resulting from crosses of the type $s_u\ s_u\ w_x\ w_x$ ♀ × $S_u\ s_u\ W_x\ w_x$ ♂ were chosen and all small and defective grains removed. The seed was then divided into 7 lots of 4 samples each representing the four respective phenotypes. Except in the first lot where three of the classes were represented by 70 seeds each, the samples comprised 45 grains.

The seeds were soaked for 24 hours in tap water at room temperature; all but the first set were then planted in wooden boxes in fine sand of approximately optimum moisture content. Each box contained 45 seeds of each of the four respective classes of seeds. The boxes were then weighed and placed in an incubator maintained at 28°C. The seedlings were kept in darkness throughout their development and the moisture lost was replaced daily.

The first samples were taken after the 24 hour period of soaking and subsequent samples at the intervals indicated in tables 13 and 14 where

TABLE 13

Dry weights in grams of seed residues during seedling growth. The values are adjusted to a basis of 45 individuals.

TIME IN DAYS	CLASS OF SEEDLING			
	S_uW_x	S_uw_x	s_uW_x	s_uw_x
0	8.05	8.04	6.86	6.92
1	8.06	8.16	6.79	6.45
2	7.44	7.21	5.96	5.82
3	5.94	6.00	4.57	4.09
S_u, 5; s_u 4.3	3.97	3.56	2.62	2.62
S_u, 6; s_u, 5.3	3.04	2.85	2.18	2.05
S_u, 7; s_u, 6.3	2.00	1.93	1.32	1.30

TABLE 14

Dry weights in grams of sprouts during seedling growth. The values are adjusted to a basis of 45 individuals.

TIME IN DAYS	CLASS OF SEEDLING			
	S_uW_x	S_uw_x	s_uW_x	s_uw_x
0	1.17	1.18	1.39	1.38
1	1.23	1.25	1.44	1.46
2	1.68	1.81	2.00	1.87
3	2.62	2.71	2.75	2.73
S_u, 5; s_u, 4.3	3.86	4.16	3.79	3.65
S_u, 6; s_u, 5.3	4.50	4.57	4.08	3.74
S_u, 7; s_u, 6.3	4.91	5.06	3.92	4.03

the data relating to the experiment are summarized. The sprouts were separated from the seed residues while the seedlings were fresh; in this condition the operation is performed clearly and with ease. The material was then dried for about 20 hours at 100°C and weighed after cooling in a dessicator.

The germination was very satisfactory. In 23 of the 24 separate samples, all of the seeds or all but one sprouted. The twenty-fourth sample, being

the s_u w_x seeds in the last lot (6–1/3 days), comprised only 40 healthy seedlings. In the accompanying tables the weights of the dry substance have been adjusted to a uniform basis of 45 seedlings.

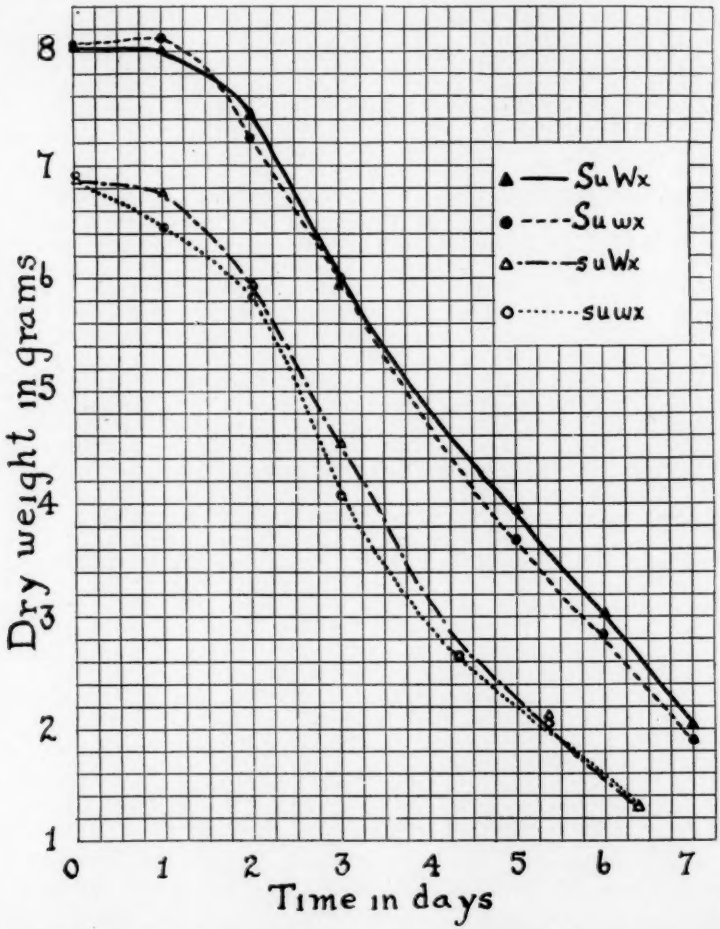


FIGURE 5.—Decreases in dry weight of seed residue due to digestion of endosperm reserves during seedling development.

The changes in weight of the seed residues and the rates of sprout development are easily followed from the graphs shown in figures 5 and 6.

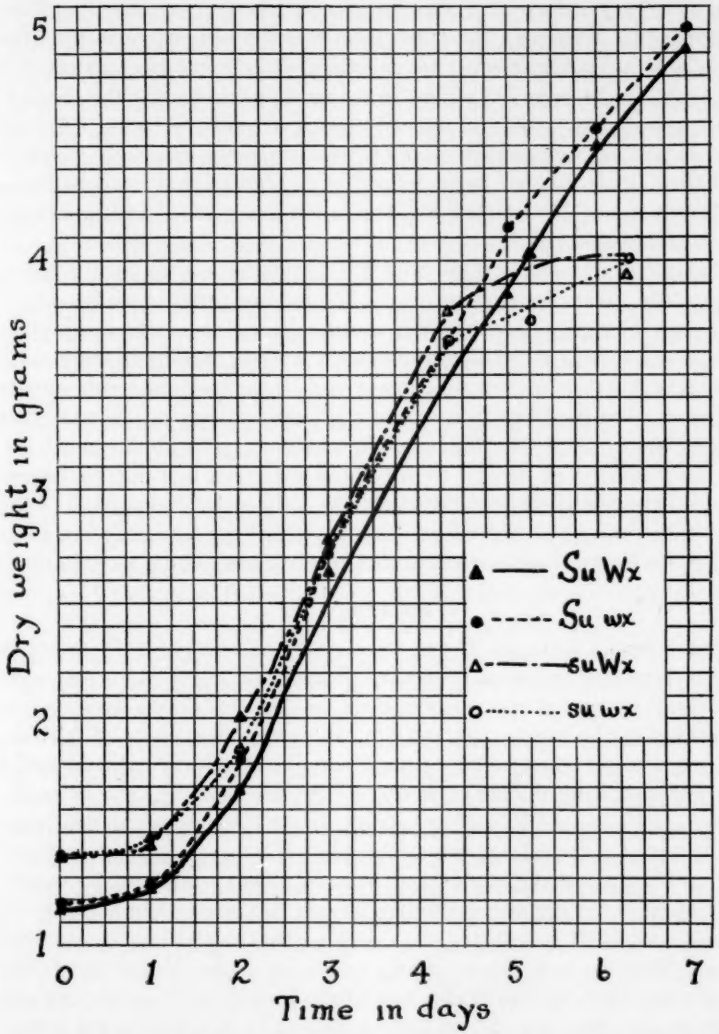


FIGURE 6.—Increase in dry weight of $S_u\ W_x$, $S_u\ w_x$, $s_u\ W_x$ and $s_u\ w_x$ sprouts during seedling growth.

As indicated in figure 5 the initial weights of the sugary seed residues are about 15 percent less than those of the non-sugary class; the waxy

and non-waxy values for each of these groups, on the other hand, are only slightly different. The question in which we are particularly interested is whether the reserves are consumed at different rates by the four classes of seedlings and this may be answered by comparing the slopes of the curves in the figure. In the early stages of seedling development the two sugary classes decrease somewhat more rapidly in weight, perhaps, but the difference is not large; thereafter the rates are as nearly alike as one could reasonably expect if the four curves represented random samples of the same material.

Table 14 and figure 6 show the course of sprout development. Up to 4 days the curves for all four classes of seed run closely parallel. After this time the rate of sprout growth in the sugary class breaks sharply but the $S_u\ W_x$ and $S_u\ w_x$ types continue with little slackening in speed until the seventh day when the experiment was terminated. It would appear therefore, that in the early stages of seedling development that the four classes of sprouts $S_u\ W_x$, $S_u\ w_x$, $s_u\ W_x$ and $s_u\ w_x$ grow at the same rate.

I am indebted to Mr. C. R. BURNHAM for much assistance rendered in the course of these investigations and for general supervision of the field work during the summer of 1926. My thanks are also due Mr. F. A. ABEGG for his painstaking work in making the chemical analyses reported.

DISCUSSION

The breeding facts relating to the sugary and the waxy characters in maize show that pollen grains which are identical so far as nuclear composition is concerned may differ in their capacity for further development according to the genetic character of the parent sporophyte. This conclusion is amply borne out by the difference in the waxy ratio following the application to double recessive individuals ($s_u\ s_u\ w_x\ w_x$) of pollen from $s_u\ s_u\ W_x\ W_x$ and $S_u\ s_u\ W_x\ w_x$ plants. In the former case the deficiency in the waxy ratio is large (about 15 percent); the double heterozygote ($S_u\ s_u\ W_x\ w_x$), on the other hand leads to a waxy ratio in the sugary class which is not significantly different from that given when $S_u\ S_u\ W_x\ w_x$ pollen is used. It is not the fact, therefore, that the male gametophytes arising from the $s_u\ s_u\ W_x\ w_x$ plants carry the sugary factor that is the significant thing, for this is also true of the "regularly" behaving sugary pollen grains from $S_u\ s_u\ W_x\ w_x$ individuals but, rather, that in the former case the $s_u\ W_x$ and $s_u\ w_x$ pollen grains are produced by a sporophyte homozygous for sugary and in the latter by a plant carrying one dose of the dominant non-sugary gene.

The development of the male gametophyte is governed in part, therefore, by the character of the extra-nuclear organization with which the parent sporophyte endows it and in part by its own particular factorial composition. Both these influences are manifested in the material under consideration. The waxy gene and its allelomorph occasion a differential effect in the gametophyte directly. There is clear-cut evidence for this statement aside from the matter of pollen tube growth. As the iodine test shows, the waxy factor modifies the character of the starch laid down in the microspore. Segregating plants produce two visibly different classes of pollen grains, in equal numbers, corresponding to the respective parental types. The sugary gene acts only indirectly on the haploid individuals through changes effected during development of the parent sporophyte. The effects of the sugary gene are carried over from the diploid plant to the pollen as a substratum, so to speak, upon which waxy and non-waxy gametophyte nuclei react very differently. The primary action of the sugary gene does not originate in the gametophyte as in the waxy case.

In the present paper we have been concerned with the mechanism of the change in the waxy ratio brought about by the sugary factor. What is the character of the handicap imposed by this gene and at what point does it operate? Counts on pollen from $s_u\ s_u\ W_x\ w_x$ plants show equal numbers of the two expected classes of grains, which proves that reduction proceeds normally. It might be supposed that there is a differential mortality between $s_u\ W_x$ and $s_u\ w_x$ gametophytes during the free pollen grain stage. This is improbable. In its non-vegetative phase one would expect the microgametophyte to be least subject to the action of selective forces. Maize pollen contains a relatively high proportion of readily available food materials, however, and it is conceivable that this supply becomes reduced through respiration more rapidly in sugary-type pollen grains. But chemical analyses reveal no important differences in content of water-soluble carbohydrates in the four respective classes of pollen.

While critical evidence for the view is not at hand it appears most probable that waxy and non-waxy pollen tubes descended from $s_u\ s_u\ W_x\ w_x$ plants grow at significantly different rates during the initial period of development in the style. We have suggested that this situation prevails as long as the reserves contributed by the pollen are being utilized. Subsequently when the two classes of gametophytes become dependent upon the food materials in the style they elongate at the same rate. There are two facts which point to this conclusion. It has been shown (BRINK and BURNHAM 1927) that the composition of the pistillate parent with

respect to the S_u, s_u pair does not influence the waxy ratio. This suggests that the selective effect is worked out by the pollen tube independently of any corresponding relationship in the style. Conclusive evidence is given in the present communication for the view that the deficiency of waxy seeds following matings in which pollen from $s_u\ s_u\ W_x\ w_x$ plants is employed cannot be due to a *constant* difference in rate of growth of $s_u\ W_x$ and $s_u\ w_x$ male gametophytes. If these two classes of pollen tubes from sugary plants elongate at constantly different rates we would expect fewer and fewer waxy seeds as we pass from the tip to the butt of the ear. Such a result should ensue, of course, as a result of the varying distances the competing tubes must travel to reach ovaries at different levels.

On a series of 20 ears, resulting from combinations of the type $S_u\ S_u\ w_x\ w_x$ ♀ $\times s_u\ s_u\ W_x\ w_x$ ♂, we have classified the seeds in the upper and lower halves of the ears separately. The net proportion of waxy seeds for the entire group is 20.25 percent. This is nearly 30 percent in defect of Mendelian expectation and we would expect, if the discrepancy were due to a constantly lower rate of growth of waxy pollen tubes, few if any waxy seeds in the lower half of the ear. The counts show, however, just as high a proportion of waxy grains in the butt region as in the upper portion. As a matter of fact there are 1.69 percent more but the difference is not significant. The absence of a graded distribution of the two classes of seed on the ear definitely shows that the immediate cause of the abnormal ratio is not a *constant* difference in rate of growth of waxy and non-waxy pollen tubes. This conclusion also applies, probably, to the deficiency of waxy seeds in stocks not involving the sugary gene discussed elsewhere (BRINK 1925).

The hypothesis that the selective action occurs during the initial period of pollen tube growth conforms with the fact that waxy and non-waxy gametophytes at this stage are dependent upon different types of starch reserves as the iodine test reveals. The physiological changes which the sugary gene calls forth, however, leading to the wider discrepancy in the waxy ratio have thus far eluded us. There are differences in chemical composition of the pollen from $S_u\ S_u\ W_x\ W_x$, $S_u\ S_u\ w_x\ w_x$, $s_u\ s_u\ W_x\ W_x$ and $s_u\ s_u\ w_x\ w_x$ plants but these do not bear any evident relation to the varying potentialities for development of the four classes of gametophytes. If we may judge from the action of the sugary and waxy genes in the endosperm the double recessive combination, $s_u\ w_x$, does not decrease in a disproportionate way the amount of reserve food synthesized. There is a relatively large difference in the weight of sugary and non-sugary endosperms but within each of these classes the waxy gene has about the same effect so far as amount of stored substance is concerned.

Studies on rate of growth of sugary and non-sugary seedlings do not reveal any striking difference in the behavior of waxy and non-waxy individuals as between these classes. The endosperm reserves in the sugary waxy ($s_u\ w_x$) seed are as readily utilized and the sprouts grow as rapidly as those of the other three types. The conditions governing rate of growth of the seedling may not be comparable, however, to those in the pollen tube and the fact that $s_u\ w_x$ sprouts do not lag behind the others does not necessarily invalidate our hypothesis.

The distribution of seeds following the application to $s_u\ s_u\ w_x\ w_x$ stocks of mixtures of pollen from $S_u\ S_u\ W_x\ w_x$ and $s_u\ s_u\ W_x\ w_x$ plants indicates that not only are $s_u\ w_x$ gametophytes from sugary homozygotes handicapped but also $s_u\ W_x$ pollen tubes as compared with the corresponding non-sugary classes. It appears, therefore, that the sugary gene in homozygous condition in the sporophyte depresses the initial rate of growth of all gametophytes arising from it; if the pollen is of the waxy type the effect is more marked.

All those who have given close attention to the matter have noted that following combinations of the types $W_x\ w_x$ selfed, or $w_x\ w_x\ ♀ \times W_x\ w_x\ ♂$ the variations in the proportion of waxy seeds from ear to ear are significantly greater than should result from chance alone. MANGELSDORF and JONES (1926) in considering this situation broach the idea that the deviations in the waxy ratio may not be due to the waxy gene at all but to a factor linked with it. In other words, it is only the circumstance that the waxy gene occupies a locus in chromosome I adjacent to a particular factor affecting pollen tube growth that is responsible for the frequently observed irregular waxy ratios. There are some breeding results which might suggest such a relationship but it seems to us that this view fails to give due weight to one of the most definitely established facts we have regarding the waxy gene, namely, that it occasions a clear-cut differential effect in the gametophyte which may be demonstrated at the pollen grain stage by the iodine test. Moreover, since the action is on the food reserves which are directly utilized in pollen tube formation it is just the sort of change which might lead to an altered rate of development of the gametophyte.

There is little doubt that much of the variation in the waxy ratio commonly observed has a factorial basis and it may be that one of the principal modifying genes is linked with waxy. The case which we mentioned earlier (BRINK, 1925, p. 367) suggesting the presence of a linked modifier has been intensively investigated since. The results, however, are difficult to interpret; the deviations from Mendelian expectation are usually so

small that their significance cannot be established on account of the inadequate numbers which single ear populations afford. The behavior of one of our sugary stocks segregating for waxy gives some indication of a gene linked with waxy which modifies pollen tube growth but the analysis has not been carried far enough to warrant more than mention at present.

The disturbance in the waxy ratio occasioned by the sugary gene affords substantial evidence for the conclusion that it is the waxy factor itself and not a gene linked with it which is primarily responsible for modification of the Mendelian distribution. Very frequently the deviation in the waxy ratio on single ears in sugary stocks is large enough to be statistically significant obviating the difficulties which arise in classifying non-sugary individuals with respect to departures from the normal value. We have records on 128 different matings involving three distinct stocks in which staminate parents of the type $s_u\ s_u\ W_x\ w_x$ have been used. In three of the cases only does the proportion of waxy seeds exceed expectation on the Mendelian basis and in none of these is the deviation greater than twice its probable error. If the sugary gene were interacting with a gene linked with waxy rather than with the latter factor itself we should expect the waxy ratios to be grouped around three modes, a high, a low and a normal. But the fact is that, with the exception of the three ears mentioned, the ratios are all low. This regularity of behavior argues for the view that the primary differential is actually the waxy gene.

The findings in regard to the effect of the sugary gene on the waxy ratio show, further, that a factor may modify pollen tube growth without acting as a differential in the gametophyte. We do not know to what extent the variations in the waxy ratio are due to such genes but it would be surprising, indeed, if sugary stood alone in this class.

In a former paper (BRINK 1925) the suggestion was offered that the genes most likely to modify pollen tube development are those concerned in carbohydrate transformations. This was not a random observation nor was the statement based wholly upon the meager statistics relating to established cases of differential pollen tube growth. The hypothesis rests upon considerations which merit more attention, we believe, than they appear to have received.

Pollen tube development, according to the author's view, involves growth processes not essentially different from certain of those occurring in the diploid plant. A group of chemical reactions is undoubtedly common to both gametophyte and sporophyte and there would seem to be no valid reason why these particular transformations may not be governed

by the same genes in both cases. The assumption that factors specifically affecting pollen tube growth are a class unto themselves rests upon a point of view for which there is no more justification, in our opinion, than for the outworn conception of unit-characters. Perhaps our static theory of heredity is mainly responsible for this attitude. We relate genes to certain end results rather than to the processes involved in the production of these; and, incidentally, we find ourselves in difficulties when some accessory circumstance plays a preponderating rôle. We associate a factor with a pronounced change in one part and discountenance the possibility that it may operate elsewhere even though without a readily discernible effect. Unknowingly, perhaps, we allow a nomenclature founded on conspicuous morphological effects to circumscribe our ideas.

In such a plant as maize whose style often attains a length of 20 cm the pollen tube develops a limiting membrane which in comparison with the size of the initial microspore is enormous. The fully developed microgametophyte is largely pollen tube wall so far as bulk of tissue is concerned. The pollen grain is, roughly, 0.1 mm in diameter; the length of the pollen tube is frequently 2000 times this value. Since the wall consists of a cellulose-like substance a considerable amount of carbon is required in its formation. This carbon is undoubtedly derived in large part from the sugars in the style and the reactions involved in their utilization would appear to be the most important in pollen tube development.

The pollen tube protoplast at no time becomes large but advances with the tip of the elongating tube, the older parts of which are cut off at frequent intervals by cross walls. If there is no actual increase in the mass of protoplasmic substance, as seems probable in those cases where the sperm nuclei are formed before the pollen germinates, the initial supply of nitrogenous material derived from the grain may serve for the complete development of the gametophyte. This may apply also to the other components of the protoplasm.

According to this view pollen tube growth consists essentially in the development of a means of transporting a fully organized protoplast from the pollen grain to the embryo sac. This is accomplished by the elaboration of a hollow cellulose structure, the pollen tube proper, through which the tri-nucleate protoplast passes. The metabolic activity of the gametophyte centers around the processes involved in building up the pollen tube wall. The wall itself is a variety of cellulose and the materials available for its construction in the style and in the pollen are principally sugars and starch and in some species, fats. Is it not logical to assume

that the genes most likely to modify pollen tube growth are some of those which control the metabolism of these particular substances?

SUMMARY

1. In the foregoing paper the results of some further experiments relating to the anomalous action of the sugary gene on the waxy ratio are discussed.

2. The marked deficiency of waxy seeds, as compared with Mendelian proportions, resulting when pollen from $s_u\ s_u\ W_x\ w_x$ plants is used cannot be accounted for on the basis of irregularities at the reduction divisions; counts of pollen mounted in the iodine reagent show equal numbers of waxy and non-waxy grains.

3. A study of the distribution of waxy and non-waxy seeds on a series of ears resulting from crosses of the type $s_u\ s_u\ w_x\ w_x$ ♀ × $s_u\ s_u\ W_x\ w_x$ ♂ reveals no significant difference between the upper and lower halves. This shows that the deficiency of waxy seeds in such cases is not due to a *constantly* different rate of growth of $s_u\ W_x$ and $s_u\ w_x$ pollen tubes.

4. Proximate chemical analyses of pollen from $S_u\ S_u\ W_x\ W_x$, $S_u\ S_u\ w_x\ w_x$, $s_u\ s_u\ W_x\ W_x$ and $s_u\ s_u\ w_x\ w_x$ plants do not show differences which bear any evident relationship to the developmental capacities of the male gametophytes arising from these individuals.

5. Investigations on the relative weights of the four classes of seed on ears segregating for sugary and waxy demonstrate that the double recessive combination, sugary waxy, does not have a disproportionate effect on the amount of reserves synthesized.

6. During the development of the seedling the endosperm reserves in the sugary waxy seeds are as readily utilized and the sprouts grow as rapidly as those of the other three types, $S_u\ W_x$, $S_u\ w_x$, and $s_u\ W_x$.

7. Experiments with mixtures of pollen from $S_u\ S_u\ W_x\ w_x$ and $s_u\ s_u\ W_x\ w_x$ plants show that both $s_u\ W_x$ and $s_u\ w_x$ pollen tubes are handicapped in development as compared with the corresponding non-sugary classes; the waxy-type pollen is affected in a significantly greater degree.

8. The hypothesis we have outlined earlier seems best to account for the modifying action of the sugary gene on the waxy ratio. It is assumed that the cytoplasm contributed by the sporophyte to the microspores is different in sugary and non-sugary plants; in the presence of sugary-type cytoplasm waxy male gametophytes are markedly handicapped as compared with the non-waxy class. Sugary-type cytoplasm appears to increase the disparity in developmental capacity which "normally" exists between waxy and non-waxy pollen tubes.

9. Direct evidence for the view that the selective action occurs during the stage in which the pollen reserves are being utilized is not yet available but this conception seems to best fit the facts at hand.

10. In the light of the present evidence it appears probable that the deficiency of waxy seeds usually observed in non-sugary stocks is also due to differential development during the initial stages of pollen tube growth.

LITERATURE CITED

BRINK, R. A., 1925 Mendelian ratios and the gametophyte generation in angiosperms. Genetics **10:** 359–394.

BRINK, R. A., and BURNHAM, C. R., 1927 Differential action of the sugary gene in maize on two alternative classes of male gametophytes. Genetics **12:** 348–378.

MANGELSDORF, P. C., and JONES, D. F., 1926 The expression of Mendelian factors in the gametophyte of maize. Genetics **11:** 423–455.

SAX, KARL, 1921 Simple device for weighing seeds. Bot. Gaz. **71:** 399. *1 fig.*

A FACTOR FOR YELLOW-GREEN CHLOROPHYLL COLOR IN MAIZE AND ITS LINKAGE RELATIONS[1]

MERLE T. JENKINS[2]

United States Department of Agriculture, Washington, D.C.

Received April 4, 1927

TABLE OF CONTENTS

	PAGE
INTRODUCTION	492
Description of yellow-green plants	492
Inheritance of yellow-green chlorophyll color	493
Linkage relations of Y_g y_g	494
Methods of calculating the percentage of crossing over in F_2 progenies	495
Linkage between Y_g y_g and *Cc*	497
Linkage of Y_g y_g with S_h s_h and W_x w_x	500
Linkage between Y_g y_g and S_h s_h	501
Linkage between Y_g y_g and W_x w_x	501
Order of genes $Y_g-C-S_h-W_x$	502
SUMMARY	504
LITERATURE CITED	504
APPENDIX	505

INTRODUCTION

Chlorophyll variations, both in the seedlings and in the mature plants, have been found very commonly in maize. The recent extensive use of self-fertilized lines as a basis for corn improvement has aided in bringing to light large numbers of these variations. The occurrence of a large number of chlorophyll defects of maize in progenies from ears self-fertilized for the first time in 1922 in connection with the corn breeding experiments at Ames, Iowa, has been reported previously (JENKINS 1924). One of these progenies, from Walden Dent corn, segregated for normal-green and yellow-green plants. The mode of inheritance of this character and the linkage relations of the determining gene are reported here.

DESCRIPTION OF YELLOW-GREEN PLANTS

The yellow-green character is apparent and ordinarily may be recognized easily both in the seedlings and in the mature plants. The yellow-

[1] Joint contribution from the Office of Cereal Crops and Diseases, Bureau of Plant Industry, UNITED STATES DEPARTMENT OF AGRICULTURE, and the Department of Farm Crops and Soils, IOWA AGRICULTURAL EXPERIMENT STATION.

[2] Associate Agronomist in Corn Investigations, Office of Cereal Crops and Diseases, Bureau of Plant Industry.

green seedlings vary somewhat in color with age and with different conditions of temperature and light. When they first emerge they generally are yellowish in color, ranging from Baryta yellow to Pinard yellow (RIDGWAY 1912). As they grow older a small amount of chlorophyll gradually develops. Usually the chlorophyll begins to develop normally at the tips of the leaves and gradually extends backward toward the base. Chlorophyll continues to develop slowly until the plants become approximately a javel green (RIDGWAY 1912). With slight variations, this color is retained until maturity.

Mature yellow-green plants are very weak. Many of them mature seed, but they have been so late in silking and tasseling that it has been difficult to obtain crosses between them and other stocks.

INHERITANCE OF YELLOW-GREEN CHLOROPHYLL COLOR

The original progeny segregating for yellow-green plants consisted of 103 green and 39 yellow-green plants. This ratio indicates that the yellow-green plants were simple Mendelian recessives. The factor pair concerned has been designated by the symbols Y_g y_g. Six of the green plants in the original progeny were self pollinated. The following year seed was planted from one of the 6 selfed ears that was known to be segregating for yellow-green plants, and 7 green plants were selfed.

TABLE 1

Number of green and yellow-green plants in the progenies of self-fertilized green plants in families segregating for yellow-greens.

PROGENY NUMBER	NUMBER OF PLANTS	
	Green	Yellow-green
894*	103	39
895	81	26
896	108	51
1997	151	46
1998	140	40
2000	138	40
2002	118	37
2003	145	55
2005	134	35
2007	187	60
Total	1305	429
Expected (3:1 ratio)	*1301*	*433*

Dev.÷P. E.=.38

* Original ear from a self-pollinated plant of Walden Dent corn.

In all, 13 green plants from segregating progenies were self pollinated. Four of these produced progenies of green plants only, and 9 produced segregating progenies. This is a deviation of only $.3 \pm 1.1$ from the expected 1:2 ratio. The data on the 9 segregating progenies of the self-fertilized green plants, and also those on the progeny of the original plant, 894, are shown in table 1. These 10 segregating progenies had a total of 1,305 green plants and 429 yellow-green plants. In this case Dev. $\div$ P.E. $= .38$.

One of the yellow-green plants in the original progeny also was self pollinated, and a cross was made between two of the yellow-green plants. The progeny of this self-fertilized yellow-green plant and that of the cross between two yellow-green plants consisted of 86 yellow-green plants and no green plants.

Additional data on the segregation for yellow-green plants were obtained and are reported in connection with the linkage studies. In the total of 120 progenies segregating for $Y_g\ y_g$ (tables 4, 7, 8, 18, 20, and 22), there were 17,473 green and 5,884 yellow-green plants. The deviation from the numbers expected is equal to the probable error. The values from tables 18, 20, and 22 that were used in obtaining this total are the totals after correction for the poor germination of the s_h seeds, as explained later in connection with the linkage studies. These data are in agreement with those given in table 1 and verify the fact that yellow-green, $y_g\ y_g$, behaves as a simple Mendelian recessive to normal $Y_g\ Y_g$.

LINKAGE RELATIONS OF $Y_g\ y_g$

Studies have shown $Y_g\ y_g$ to be linked with genes located on chromosome I (LINDSTROM 1923). Other genes in this group are $C\ c$, a factor pair which determines aleurone color when the A and R factors are present, $I\ i$, a factor pair for dominant white aleurone, $S_h\ s_h$, a factor pair involved in a shrunken type of endosperm, and $W_x\ w_x$, a factor pair which determines a difference in the chemical composition of the endosperm. Tests to determine the linkage of $Y_g\ y_g$ have been conducted with $C\ c$, $S_h\ s_h$, and $W_x\ w_x$. No crosses have been made yet with the $I\ i$ factor pair.

HUTCHISON (1922) has given the linkage relations of $C\ c$, $S_h\ s_h$ and $W_x\ w_x$ indicated in figure 1. His values have been taken for the crossover percentages between these genes. DEMEREC (1924) has shown that virescent 1, $V_1\ v_1$, also belongs on this chromosome and probably is located to the right of $W_x\ w_x$.

The early data involving $Y_g\ y_g$ and two or more of the other genes on chromosome I seemed to indicate that $Y_g\ y_g$ was situated to the left of $C\ c$. Effort has been concentrated, therefore, on proving definitely whether

$Y_g\ y_g$ is located to the left of $C\ c$ and on determining as precisely as possible the exact distance between these two genes.

Unfortunately, in the linkage studies with $Y_g\ y_g$ it has not been possible thus far to utilize backcrosses extensively. As mentioned, yellow-green plants are very weak and blossom late in the season. Even by starting

C	S_h	W_x
c	s_h	w_x
0	3.5	21.9

FIGURE 1.—Linkage relations of the factors C_c, S_h s_h and W_x w_x (after HUTCHISON 1922).

plants in the greenhouse and giving them the best of care, it has been possible to have only two plants available for backcrossing. The rest either have died or have shed pollen too late to be of use.

Methods of calculating the percentage of crossing over in F_2 progenies

EMERSON'S formulas (1916), together with two extensions, have been used in calculating the percentage of crossing over directly from the F_2 ratios. The formulas proposed by EMERSON (1916) were used in computing the percentage of crossing over in all progenies involving only dihybrid ratios. These formulas for r and s are,

$$r = .5\sqrt{E - M} \tag{1}$$

$$s = .5\sqrt{E + M} - r \tag{2}$$

where E represents the two extreme, and M the two middle classes of an F_2 distribution.

Some of the F_2 progenies segregated for two aleurone factor pairs, $C\ c$ and $R\ r$, and the chlorophyll factor pair $Y_g\ y_g$ (tables 4, 8, 20, and 21). These gave 9:7 ratios for aleurone color and 3:1 ratios for chlorophyll color, with evidence of linkage between $C\ c$ and $Y_g\ y_g$. Other F_2 progenies (table 9) segregated for the aleurone factor pair $C\ c$ and two chlorophyll factor pairs, $Y_g\ y_g$ and $V\ v$. Since the virescent and yellow-green plants could not be separated accurately they were treated as one non-green class. This gave a 3:1 ratio for aleurone color and a 9:7 ratio for chlorophyll color, again with evidence of linkage between $C\ c$ and $Y_g\ y_g$. Both of these combinations gave modified 27:9:21:7 ratios.

The formulas used with these data were those suggested by BRUNSON (1924) for use with trihybrid ratios involving two complementary genes,

one of which is linked with a third gene. The formulas for calculating the values of r and s given by BRUNSON are:

$$r=\sqrt{\frac{(AB+3ab)-(Ab+aB)}{18}} \tag{3}$$

$$s=1/4\sqrt{AB+Ab+aB+ab}-r \tag{4}$$

where, in this case,

AB = Purple aleurone, green plants
Ab = Purple aleurone, non-green plants
aB = Colorless aleurone, green plants
ab = Colorless aleurone, non-green plants.

Still other F_2 progenies (tables 5, 6, and 10) segregated for the two aleurone factor pairs, $C\ c$ and $R\ r$, and also for the two chlorophyll factor pairs, $Y_g\ y_g$ and $V\ v$. This gave a 9:7 ratio for aleurone color and a 9:7 ratio for chlorophyll color, with evidence of linkage between one of the chlorophyll and one of the aleurone factors. The F_2 distribution, therefore, was a modified 81:63:63:49 ratio. An adaptation of EMERSON'S (1916) formulas suitable for a tetrahybrid ratio involving two pairs of complementary genes, with linkage between one of the genes in each pair was developed for calculating the percentage of crossing over in these progenies.

If any gametic series between two linked factors is represented as $r:s:s:r$, then the expected F_2 tetrahybrid distribution in the four phenotypic classes may be represented as follows:

$AB = 27\ r^2+18\ (2\ rs+s^2)$ Purple aleurone, green plants
$Ab = 9\ r^2+18\ (2\ rs+s^2)$ Purple aleurone, non-green plants
$aB = 9\ r^2+18\ (2\ rs+s^2)$ Colorless aleurone, green plants
$ab = 19\ r^2+10\ (2\ rs+s^2)$ Colorless aleurone, non-green plants.

Expressing the values of r and s in terms of the phenotypic classes, it follows from the above equations that

$$288r^2=9(AB+ab)-7(Ab+aB)$$

Then

$$r=\sqrt{\frac{9(AB+ab)-7(Ab+aB)}{288}} \tag{5}$$

Also, from the same equations,

$$64(r^2+2rs+s^2)=AB+Ab+aB+ab$$

Then

$$8(r+s)=\sqrt{AB+Ab+aB+ab}$$

And

$$s=1/8\sqrt{AB+Ab+aB+ab}-r \qquad (6)$$

These equations are equally applicable to coupling ($r>s$), repulsion ($r<s$), and to independent inheritance ($r=s$).

Linkage between Y_g y_g and C c

The first indications of linkage between yellow-green chlorophyll color and aleurone color came from the F_2 progenies of three crosses made in 1923. The genetic constitution of the parental plants of these crosses is shown below:

Cross number 1.—*Y_g Y_g AA Cc Rr × y_g y_g AA cc rr*
Cross number 2.—*y_g y_g VV AA cc rr × Y_g Y_g vv AA CC Rr*
Cross number 3.—*Y_g Y_g vv AA CC Rr × y_g y_g VV AA cc rr*

Each of the crossed ears had seeds with purple aleurone and seeds with colorless aleurone. Purple seeds from each ear were planted in 1924 and many of the resulting plants were self pollinated. Since the purple seeds from each ear were heterozygous for *C c* and *R r*, all of the F_2 progenies segregated into 9:7 ratios for aleurone color. When plants were grown from these kernels linkage was shown in each F_2 progeny between y_g and one of the factors for aleurone color. The aleurone factor concerned in this linkage has been determined since to be the *C* factor. The data on the F_2 progenies from purple seeds from cross number 1 are shown in table 4. The F_1 plants from these seeds were heterozygous for *Y_g C R · y_g c r*. They therefore gave modified 27:9:21:7 ratios in the F_2. Formulas 3 and 4 were used in calculating the crossover percentages. The average crossover percentage was 22.0.

Data on the F_2 progenies of the purple seeds of cross number 2 are shown in table 5. The F_1 plants from these seeds were heterozygous for *y_g c r V · Y_g C R v*. As the yellow-greens and virescents could not be separated accurately, they were grouped into one non-green class. The F_2 distribution therefore was a modified 81:63:63:49 ratio. Each F_2 progeny showed indications of linkage between one of the chlorophyll and one of the aleurone factors. The virescent factor, *v*, came into the cross with purple aleurone, and y_g came in with colorless aleurone. Since each of the

progenies contained an excess of non-greens among the plants from seeds with colorless aleurone and a deficiency of non-greens among the plants from seeds with purple aleurone, it is evident that y_g must be the chlorophyll factor concerned. Formulas 5 and 6 were used in calculating the crossover percentages in the F_2 progenies from this cross. Data from all of the progenies from this cross gave an average crossover percentage of 21.3. This is in very close agreement with the value obtained for the progenies of cross number 1.

The F_1 plants from purple seeds from cross number 3 (table 6) were heterozygous for Y_g *C R v* · y_g *c r V*. The virescent plant which was the pistillate parent of this cross was heterozygous for defective seeds. Of the 22 F_2 progeny ears obtained, 11 segregated for defective seeds. The factor for defective seeds apparently was linked with *v*. As the defective seeds germinated poorly this influenced the chlorophyll ratios also. For this reason data on the progenies segregating for defective seeds have been omitted from table 6, in which the data on the other progenies of cross number 3 are given. Crossover percentages for the F_2 progenies of cross number 3 were calculated in the same manner as for cross number 2.

The totals for the 11 F_2 progenies shown indicate an average crossover percentage of 15.7. This is considerably lower than 22.0 and 21.3, the values obtained for crosses 1 and 2, respectively. The difference between the observed numbers and those expected with 22.0 percent of crossing over is more than five times the probable error (table 6). It may be that genetic factors which influence the percentage of crossing over are present in this material. The wide differences in the crossover values obtained for the individual progenies, which range from 22.4 to 5.7 percent (table 6) would tend to support this view.

If genetic factors modifying the percentage of crossing over are involved in cross number 3, it should be possible to isolate them and obtain strains which behave regularly but differently in this respect. With this in mind, the F_2 progenies of this cross were carried into the F_3 and a few of them into the F_4 in an effort to select lines which differed uniformly in their percentages of crossing over between *C c* and Y_g y_g. Two F_2 progenies of cross number 1 were carried into the F_3 for comparison.

It has not been possible to draw definite conclusions from the results obtained as to the existence of genetic factors modifying the percentage of crossing over in this material. The data consequently are shown in tables 7, 8, 9, and 10 only to give additional information on the percentage of crossing over between *C c* and Y_g y_g.

Of the 33 F_3 and F_4 progenies which segregated for $Y_g\ y_g$ and $C\ c$ only (table 7), 27 involved coupling and 6 repulsion. The 27 progenies showing coupling had an average crossover percentage of 19.7. The 6 progenies showing repulsion had a crossover percentage of 25.9.

Of the 26 F_3 and F_4 progenies segregating for $Y_g\ y_g$, $C\ c$, and $R\ r$ (table 8), all but two showed coupling. They had an average crossover percentage of 20.6. The two progenies showing repulsion had an average crossover value of 20.5 percent.

Of the 9 F_3 progenies segregating for $Y_g\ y_g$, $C\ c$ and $V\ v$ (table 9), 8 showed coupling and 1 showed repulsion. The 8 progenies showing coupling had an average crossover percentage of 20.7. The one progeny showing repulsion had a crossover value of 32.0 percent.

Of the 24 F_3 progenies segregating for $Y_g\ y_g$, $C\ c$, $R\ r$ and $V\ v$ (table 10), 21 showed coupling and 3 showed repulsion. The average crossover values were 21.9 percent for the 21 progenies showing coupling and 16.9 percent for the 3 showing repulsion.

A few additional data on the linkage between $Y_g\ y_g$ and $C\ c$ are available from F_2 progenies segregating also for $S_h\ s_h$ and $W_x\ w_x$ (tables 18 and 20, which are summarized in tables 19 and 21). The data in table 19 indicate a crossover percentage between $Y_g\ y_g$ and $C\ c$ of 22.4 while those in table 21 indicate a value of 17.3 percent.

Data on the progenies of two plants of the constitution $\frac{y_gC}{Y_gc}$ which were backcrossed to the double recessive are shown in table 11. Of the 126 plants obtained, 17, or 13.5 percent, were crossovers.

It seemed desirable to obtain an average value for crossover percentage in all of the F_2, F_3, and F_4 progenies. There were several different ratios and both the coupling and repulsion phases were represented. The average crossover percentage therefore was determined by dividing the sum of the values for s or r (s in the case of coupling and r in the case of repulsion) by the sum of the values for $r+s$ and multiplying the quotient by 100. This method weights the data from the different progenies having the same kind of ratio in proportion to the square root of the number of individuals in the progenies. It also gives less weight to data involving the more complex relations and greater weight to data involving dihybrid ratios, which seemed desirable.

A summary of all of the F_2, F_3, and F_4 progenies segregating for $Y_g\ y_g$ and $C\ c$ is given in table 2. The average crossover percentage calculated for all of the progenies showing coupling was 20.5. The progenies showing

repulsion had an average crossover percentage of 23.0. The average crossover percentage for all progenies was 21.3.

TABLE 2

Summary of the data for all of the F_2, F_3, and F_4 progenies segregating for $Y_g y_g$ and Cc.

PHASE OF LINKAGE	DATA FROM TABLE	TOTAL NUMBER OF INDIVIDUALS	VALUES FOR		CROSSOVER PERCENTAGE
			$r+s$	r or s[1]	
Coupling	4	8021	22.39	4.93	22.0
	5	6139	9.79	2.09	21.3
	6	3451	7.34	1.15	15.7
	7	4403	33.18	6.54	19.7
	8	4440	16.66	3.44	20.6
	9	1044	8.08	1.67	20.7
	10	3319	7.20	1.58	21.9
Sub-total		30,817	104.64	21.40	20.5
Repulsion	7	1132	16.82	4.36	25.9
	8	420	5.12	1.05	20.5
	9	147	3.03	.97	32.0
	10	806	3.55	.60	16.9
	18, 19	898	14.98	3.36	22.4
	20, 21	482	5.49	.95	17.3
Sub-total		3,885	48.99	11.29	23.0
Grand total		34,702	153.63	32.69	21.3

[1] s in case of coupling and r in case of repulsion.

The progenies reported in table 2 showing coupling include many more individuals than those showing repulsion. Moreover, crossover percentages can be determined much more precisely from data involving coupling than from those involving repulsion. It is felt, therefore, that the crossover percentage of 20.5 obtained from the progenies showing coupling probably is the more reliable estimate of the true situation.

Linkage of $Y_g y_g$ with $S_h s_h$ and $W_z w_z$

Data obtained in 1925 and 1926 from a number of F_2 and backcross progenies segregating for $Y_g y_g$ and for either $S_h s_h$ or $W_z w_z$ or for both $S_h s_h$ and $W_z w_z$ are shown in tables 12 to 23 inclusive. Tables 16, 18, 19, 20, and 21 contain data on progenies also segregating for $C c$. The actual type of segregation in each case is indicated in the table heading. In tables 18, 20, and 22, summarized in tables 19, 21, and 23, the germination of the s_h (shrunken) seeds was less than that of the S_h seeds. Before the per-

centage of crossing over could be calculated it was necessary to correct for the poor germination of the s_h seeds. This correction was made by multiplying the total values for green and yellow-green plants from shrunken seeds by the ratio of the germination of S_h seeds to that of s_h seeds.

Linkage between $Y_g\ y_g$ and $S_h\ s_h$

Data on fourteen backcrosses between plants of the constitution $\frac{y_g S_h}{Y_g s_h}$ and the double recessive are shown in table 12. From a total of 1267 progeny, 975 parental combinations and 292 non-parental combinations were obtained. This is a deviation from equality (independent inheritance) of 341.5 ± 12.0. This difference is over twenty-eight times the probable error and indicates that $Y_g\ y_g$ and $S_h\ s_h$ are linked. An average crossover percentage of 23.0 was calculated.

The data on three groups of F_2 progenies (tables 19, 21, and 23) show crossover percentages between $Y_g\ y_g$ and $S_h\ s_h$ of 28.6, 32.7, and 28.0. The totals for all of the progenies in these tables, using the corrected values, are:

Class	$Y_g S_h$	$Y_g s_h$	$y_g S_h$	$y_g s_h$	*Total*
Number of individuals	2583	1056.9	1212	89.3	4941.2

The average crossover percentage calculated from these totals is 28.6. This is significantly higher than the value of 23.0 obtained from the backcross progenies.

Linkage between $Y_g\ y_g$ and $W_z\ w_z$

Ten plants of the constitution $\frac{y_g w_z}{Y_g W_z}$ were backcrossed in 1926 to a double recessive plant. The data on the progenies of this backcross are shown in table 13. Of the total of 1395 individuals obtained, 586 or 42.0 percent were crossovers. Four plants of the constitution $\frac{y_g W_z}{Y_g w_z}$ also were backcrossed to the double recessive. The progenies of this backcross (table 14) had a total of 414 individuals, of which 158 or 38.2 percent were crossovers. In table 13 the deviation from the numbers expected with independent inheritance is nearly nine times the probable error and in table 14 the deviation is over seven times the probable error. Both

of these deviations clearly indicate linkage between Y_g y_g and W_z w_z.

In 1925, 14 plants of the constitution $\frac{y_g W_z}{Y_g w_z}$ were crossed with a plant of the constitution $\frac{y_g W_z}{y_g w_z}$. The $\frac{y_g W_z}{y_g w_z}$ plant should form two kinds of gametes in equal numbers (y_g W_z and y_g w_z) and these should unite with gametes of the $\frac{y_g W_z}{Y_g w_z}$ plants to give 8 combinations as follows:

Non-crossovers	*Crossovers*
$y_g W_z \cdot y_g W_z$	$Y_g W_z \cdot y_g W_z$
$y_g W_z \cdot y_g w_z$	$Y_g W_z \cdot y_g w_z$
$Y_g w_z \cdot y_g W_z$	$y_g w_z \cdot y_g W_z$
$Y_g w_z \cdot y_g w_z$	$y_g w_z \cdot y_g w_z$

Among the crossover combinations, the only class which could be recognized definitely was the y_g $w_z \cdot y_g$ w_z class. This class should constitute one-fourth of the total number of crossovers.

The data on the progenies of this cross are given in table 15. The total number of crossovers was estimated by multiplying the y_g w_z class by 4. While this tends to accentuate any chance variation in the y_g w_z class in the individual progenies, the total should be fairly reliable. Among a total of 1267 individuals, 520 were calculated to be crossovers. This gives a crossover percentage of 41.0. This is in close agreement with the values of 42.0 and 38.2 obtained for the backcrosses to the double recessive.

Data for several F_2 progenies from plants of the constitution $\frac{y_g W_z}{Y_g w_z}$ (tables 19, 21, and 23) gave crossover percentages of 36.7, 39.5, and 44.1. Totals for all of these progenies gave the following F_2 distribution:

Class	$Y_g W_z$	$Y_g w_z$	$y_g W_z$	$y_g w_z$	*Total*
Number of individuals	2699.9	940	1086	215.3	4941.2

The average crossover percentage calculated from these totals is 42.4. This is in close agreement with the values reported in the preceding paragraphs.

Order of the Genes Y_g–C–S_h–W_z

The data presented have shown Y_g y_g to be linked with C c, S_h s_h and W_z w_z. The crossover percentage between Y_g y_g and C c was the smallest

obtained, that between $Y_g\ y_g$ and $S_h\ s_h$ was intermediate, and that between $Y_g\ y_g$ and $W_z\ w_z$ was the largest. This indicates that $Y_g\ y_g$ is to the left of $C\ c$ and that the order of the genes therefore is Y_g–C–S_h–W_z. Additional proof of this is to be had from three groups of F_2 progenies already discussed, in which three or more factors were segregating. These data are summarized in table 3.

TABLE 3

Summary of all F_2 progenies segregating for $Y_g y_g$ and two or more other genes on chromosome I.

DATA FROM TABLE	NUMBER OF PROGENIES	CROSSOVER PERCENTAGES IN THE REGION INDICATED					
		Y_g–C	Y_g–S_h	$Y_g W_z$	C–S_h	C–W_z	S_h–W_z
19	5	22.4	28.6	36.7	2.5	24.4	21.6
21	4	17.3	32.7	39.5	9.6	26.6	15.1
23	26	..	28.0	44.1	..	..	16.9

The data from these three groups of F_2 progenies show quite different crossover values between the different genes. The data from each group, however, indicate that the order of the genes is Y_g–C–S_h–W_z.

The final proof that this is the real situation could best be had from backcrosses involving y_g and at least two of the other genes on chromosome I. Data on two small progenies from plants of the constitution $\frac{y_g C S_h}{Y_g c s_h}$ backcrossed to a triple recessive plant, $y_g\ c\ s_h$ are shown in table 16. Among a total of 126 individuals, 16 (12.6 percent) were single crossovers in the Y_g–C region, 4 (3.2 percent) were single crossovers in the C–S_h region, and 1 individual (.8 percent) was a double crossover.

Data also are available on 14 progenies which are crosses between plants of the constitution $\frac{y_g S_h W_z}{Y_g s_h w_z}$ and a plant of the constitution $\frac{y_g s_h W_z}{y_g s_h w_z}$ (table 17). As this plant was heterozygous for $W_z\ w_z$ the percentage of single crossovers for the S_h–W_z region and also the percentage of double crossovers had to be calculated in a manner similar to that already described for the data in table 15. In the case of the single crossovers for the S_h–W_z region the $y_g\ S_h\ w_z$ class could be definitely recognized, and this class should represent one-fourth of the total number of crossovers. The total number of single crossovers for this region was computed by multiplying the $y_g\ S_h\ w_z$ class by 4. The total number of double crossovers was computed from the $Y_g\ S_h\ w_z$ class in the same manner.

Of the total of 1267 individuals shown in table 17, therefore, 284 or 22.4 percent were single crossovers in the Y_g–S_h region, 300 or 23.7 percent were calculated to be crossovers in the S_h–W_z region and 32 or 2.5 percent were calculated to be double crossovers.

On the basis of the above data it seems safe to conclude, therefore, that $Y_g\ y_g$ is located to the left of $C\ c$ on the first chromosome. The crossover percentage obtained between $Y_g\ y_g$ and $C\ c$ was 20.5 (coupling progenies, table 2). Using this value and the linkage relations of $C\ c$, $S_h\ s_h$ and $W_z\ w_z$ given by HUTCHISON (1922), a new map of chromosome I may be constructed as follows:

Y_g	20.5	C	3.5	S_h	18.4	W_z
$_gy$		c		s_h		w_z

SUMMARY

A chlorophyll variation of maize, designated yellow-green (y_g), was isolated and found to behave as a simple Mendelian recessive to the normal.

The factor pair $Y_g\ y_g$ was found to be located on chromosome I, a distance of 20.5 units to the left of $C\ c$.

LITERATURE CITED

BRUNSON, A. M., 1924 The inheritance of a lethal pale green seedling character in maize. Cornell Univ. Agric. Exp. Sta. Mem. **72**: 1–22.

DEMERIC, M., 1924 Genetic relations of five factor pairs for virescent seedlings in maize. Cornell Univ. Agric. Exp. Sta. Mem. **84**: 1–38.

EMERSON, R. A., 1916 The calculation of linkage intensities. Amer. Nat. **50**: 411–420.

HUTCHISON, C. B., 1922 The linkage of certain aleurone and endosperm factors in maize, and their relation to other linkage groups. Cornell Univ. Agric. Exp. Sta. Mem. **60**: 1421–1473.

JENKINS, MERLE T., 1924 Heritable characters of maize. 20—Iojap striping, a chlorophyll defect. Jour. Hered. **15**: 467–472.

LINDSTROM, E. W., 1923 Genetical research with maize. Genetica **5**: 327–356.

1924 Complementary genes for chlorophyll development in maize and their linkage relations. Genetics **9**: 305–326.

RIDGWAY, ROBERT, 1912 Color standards and nomenclature. pp. 1–43 and Plates 1–53. (Published by the author, Washington, D.C.)

APPENDIX

TABLE 4

Data on the F_2 progenies from purple seeds from cross number 1 ($V_g\ V_g\ AA\ Cc\ Rr \times y_g\ y_g\ AA\ cc\ rr$).

PROGENY NUMBER	PURPLE ALEURONE		COLORLESS ALEURONE		TOTAL	CROSSOVER PERCENTAGE
	Green	Yellow-green	Green	Yellow-green		
1770	16	1	10	5	32	25.5
2077	147	15	69	56	287	15.6
2078	134	17	68	37	256	25.5
2079	139	19	66	57	281	15.5
2080	139	19	87	44	289	28.7
2081	172	28	103	72	375	21.9
2082	141	13	69	39	262	22.7
2083	121	16	59	45	241	18.3
2084	191	23	85	77	376	13.8
2085	177	29	100	57	363	26.7
2086	172	28	109	76	385	22.2
2087	220	27	125	85	457	20.6
2088	142	19	88	66	315	18.9
2089	135	29	85	58	307	24.9
2090	152	16	83	67	318	15.7
2091	194	18	119	73	404	22.1
2092	135	25	95	51	306	30.2
2093	164	34	92	58	348	26.4
2094	157	20	85	53	315	23.0
2095	128	22	78	54	282	22.6
2096	147	28	100	52	327	31.0
2097	125	14	59	47	245	16.4
2098	172	19	108	74	371	20.1
2099	135	24	73	55	287	20.8
2100	147	24	88	67	326	19.7
2101	132	25	71	36	264	30.3
Total	3834	552	2174	1461	8021	22.0
Expected[1]	*3923*	*589*	*2093*	*1416*	*8021*	..

Deviation $=44 \pm 28.5$. Deviation and probable error calculated according to the method suggested by LINDSTROM (1924) from the numbers of parental and non-parental combinations expected.

[1] With a crossover percentage of 22.0.

TABLE 5

Data on the F_2 progenies from purple seeds from cross number 2 ($y_o y_o VV AA cc rr \times Y_o Y_o vv AA CC Rr$).

PROGENY NUMBER	PURPLE ALEURONE		COLORLESS ALEURONE		TOTAL	CROSSOVER PERCENTAGE
	Green	Non-green	Green	Non-green		
1655	32	13	18	23	86	15.5
1656	27	11	16	25	79	11.4
2008	125	56	83	86	350	23.3
2009	173	73	96	98	440	20.4
2010	133	75	71	92	371	22.5
2011	104	37	51	65	257	11.6
2012	120	59	85	94	358	24.5
2013	127	77	70	72	346	30.2
2014	152	78	87	79	396	28.0
2015	111	50	57	70	288	17.6
2016	176	88	94	104	462	22.6
2017	156	84	98	101	439	27.4
2018	136	83	83	109	411	24.9
2019	135	64	61	85	345	20.0
2020	116	68	78	79	341	30.8
2021	146	74	63	110	393	12.8
2022	150	64	85	88	387	20.6
2023	139	84	51	116	390	12.3
Total	2258	1138	1247	1496	6139	21.3
Expected[1]	*2261*	*1192.5*	*1192.5*	*1493*	*6139*	..

Deviation = 0 ± 25.7. Deviation and probable error calculated according to the method suggested by LINDSTROM (1924) from the numbers of parental and non-parental combinations expected.

[1] With a crossover percentage of 21.3.

TABLE 6

Data on the F_2 progenies from purple seeds from cross number 3 ($Y_g Y_g vv$ AA CC Rr × $y_g y_g$ VV AA cc rr).

PROGENY NUMBER	PURPLE ALEURONE		COLORLESS ALEURONE		TOTAL	CROSSOVER PERCENTAGE
	Green	Non-green	Green	Non-green		
2048	143	65	81	83	372	22.4
2049	30	15	19	24	88	20.5
2050	113	53	63	78	307	19.2
2051	106	51	49	53	259	20.9
2056	121	43	71	71	306	17.8
2057	128	67	66	89	350	19.7
2059	162	80	69	89	400	18.0
2060	141	54	66	73	334	14.9
2061	141	63	53	88	345	10.3
2064	130	50	55	103	338	5.7
2065	149	71	47	85	352	10.2
Total	1364	612	639	836	3451	15.7
Expected[1]	*1316*	*625.5*	*625.5*	*884*	*3451*	
Deviation[2] = 0 ± 19.0						
Expected[3]	*1266*	*675*	*675*	*835*	*3451*	..
Deviation[2] = 99 ± 19.3						

[1] With a crossover percentage of 15.7.

[2] Deviations and probable errors calculated according to the method suggested by LINDSTROM (1924) from the number of parental and non-parental combinations expected.

[3] With a crossover percentage of 22.0.

TABLE 7

Data on the F_3 and F_4 progenies from plants of the constitution $Y_g y_g Cc$.

PROGENY NUMBER	Y_gC	Y_gc	y_gC	y_gc	TOTAL	CROSSOVER PERCENTAGE
			Coupling			
4364	99	16	13	20	148	22.0
4383	144	21	21	31	217	21.7
4399	63	13	11	13	100	27.9
4401	53	5	7	17	82	15.9
4412	139	15	12	31	197	14.8
4419	143	13	17	34	207	15.7
4420	34	7	8	4	53	34.1
4432	106	10	16	23	155	18.5
4444	11	3	2	2	18	33.3
4455	147	16	13	36	212	14.8
4466	113	10	11	21	155	14.6
4467	117	21	29	43	210	27.6
4488	131	23	23	33	210	25.0
4489	75	8	10	21	114	17.3
4492	95	8	6	29	138	10.7
4495	88	21	13	20	142	27.8
4496	34	4	2	7	47	13.7
4890	110	14	9	22	155	16.1
4896	136	20	22	34	212	22.3
4911	196	24	21	46	287	17.2
4912	143	12	10	37	202	11.6
4915	63	16	8	14	101	27.6
4917	231	23	28	50	332	16.8
4918	90	11	8	19	128	16.1
4921	167	23	24	40	254	20.6
4922	30	4	4	9	47	18.8
4933	168	45	28	39	280	30.8
Total	2926	406	376	695	4403	19.7
			Repulsion			
4376	115	59	53	3	230	16.1
4379	133	67	76	7	283	.. [1]
4468	75	43	27	8	153	29.2
4484	39	21	13	4	77	34.2
4931	91	29	39	3	162	40.1
4937	124	55	46	2	227	33.2
Total	577	274	254	27	1132	25.9

[1] Crossover percentage could not be calculated with the formulas used.

TABLE 8

Data on the F_3 and F_4 progenies from plants of the constitution Y_g y_g Cc Rr.

PROGENY NUMBER	PURPLE ALEURONE		COLORLESS ALEURONE		TOTAL	CROSSOVER PERCENTAGE
	Y_g	y_g	Y_g	y_g		
			Coupling			
4365	35	4	16	11	66	19.7
4367	54	8	32	19	113	25.2
4368	109	18	67	32	226	31.4
4370	111	18	61	44	234	21.2
4378	90	16	48	34	188	22.2
4380	36	5	21	8	70	34.4
4381	18	1	9	2	30	35.8
4384	146	22	83	37	288	31.6
4400	67	11	25	21	124	18.0
4415	103	10	39	28	180	17.3
4421	125	8	56	37	226	17.8
4422	61	4	30	23	118	15.1
4434	130	27	75	42	274	29.2
4435	139	13	72	56	280	16.0
4441	62	10	40	25	137	25.0
4463	49	9	17	20	95	11.9
4469	104	16	50	40	210	18.2
4471	103	12	41	43	199	10.5
4480	49	9	40	21	119	31.5
4483	154	26	76	95	351	7.7
4888	89	6	38	24	157	18.5
4889	127	19	67	44	257	22.7
4891	127	14	59	46	246	16.6
4897	126	21	67	38	252	26.7
Total	2214	307	1129	790	4440	20.6
			Repulsion			
4423	15	11	16	5	47	24.0
4477	127	70	142	34	373	20.1
Total	142	81	158	39	420	20.5

TABLE 9

Data on the F_3 and F_4 progenies from plants of the constitution $Y_0\ y_0\ Cc\ Vv$.

PROGENY NUMBER	GREEN		NON-GREEN		TOTAL	CROSSOVER PERCENTAGE
	C	*c*	*C*	*c*		
			Coupling			
4396	20	3	10	6	39	24.4
4418	62	4	32	15	113	25.2
4429	87	4	34	40	165	4.7
4446	61	9	35	26	131	19.6
4452	39	5	23	12	79	27.5
4453	131	16	61	31	239	26.2
4461	90	6	47	31	174	18.5
4481	46	6	35	17	104	31.0
Total	536	53	277	178	1044	20.7
			Repulsion			
4494	60	26	50	11	147	32.0

TABLE 10

Data on the F_2 progenies from plants of the constitution $Y_g y_g Cc Rr Vv$.

PROGENY NUMBER	PURPLE ALEURONE		COLORLESS ALEURONE		TOTAL	CROSSOVER PERCENTAGE
	Green	Non-green	Green	Non-green		
			Coupling			
4392	46	28	28	36	138	25.2
4402	62	32	36	49	179	19.2
4413	29	14	15	14	72	24.5
4417	26	18	22	32	98	25.8
4425	115	54	59	88	316	14.4
4427	79	39	36	57	211	14.3
4433	69	40	34	56	199	17.6
4437	53	41	31	54	179	24.6
4439	48	27	30	40	145	22.5
4443	88	45	34	45	212	17.6
4445	81	56	47	48	232	34.7
4454	21	9	13	15	58	18.9
4456	51	29	37	24	141	41.9
4458	78	26	34	58	196	4.6
4465	70	40	51	35	196	41.1
4472	41	17	16	17	91	16.0
4475	36	18	15	34	103	7.1
4485	70	35	50	53	208	26.1
4486	35	10	16	23	84	5.2
4487	19	11	19	15	64	42.0
4993	65	48	39	45	197	34.3
Total	1182	637	662	838	3319	21.9
			Repulsion			
4389	108	113	116	59	396	..[1]
4398	73	67	74	43	257	22.0
4442	51	28	49	25	153	45.8
Total	232	208	239	127	806	16.9

[1] Crossover percentage could not be calculated with the formulas used.

TABLE 11

Data on progenies of the backcross $\frac{y_0C}{Y_{0C}} \times \frac{y_{0c}}{y_{0c}}$.

PROGENY NUMBER	PARENTAL COMBINATIONS		NEW COMBINATIONS		TOTAL	CROSSOVERS	CROSSOVER PERCENTAGE
	Y_{0c}	y_0C	Y_0C	y_{0c}			
4234	35	35	6	5	81	11	13.6
4235	17	22	4	2	45	6	13.3
Total	52	57	10	7	126	17	13.5
Total observed	109		17				
Calculated 1:1	*63*		*63*				
Difference	46 ± 3.79						

TABLE 12

Data on progenies of the backcross $\frac{y_0S_h}{Y_{0S_h}} \times \frac{y_{0S_h}}{y_{0S_h}}$.

PROGENY NUMBER	PARENTAL COMBINATIONS		NEW COMBINATIONS		TOTAL	CROSSOVERS	CROSSOVER PERCENTAGE
	Y_{0S_h}	y_0S_h	Y_0S_h	y_{0S_h}			
4234	34	34	7	6	81	13	16.0
4235	18	20	3	4	45	7	15.6
4293	41	35	14	5	95	19	20.0
4316	15	34	13	9	71	22	31.0
4318	20	33	13	5	71	18	25.4
4319	40	43	14	9	106	23	21.7
4321	44	66	23	14	147	37	25.2
4324	37	48	16	8	109	24	22.0
4326	42	39	11	8	100	19	19.0
4328	41	39	15	13	108	28	25.9
4330	28	35	16	5	84	21	25.0
4332	29	51	24	7	111	31	27.9
4333	22	32	11	5	70	16	22.9
4334	18	37	13	1	69	14	20.3
Total	429	546	193	99	1267	292	23.0
Total observed	975		292				
Calculated 1:1	*633.5*		*633.5*				
Difference	341.5 ± 12.0						

TABLE 13

Data on progenies of the backcross $\frac{y_0w_x}{Y_0W_x} \times \frac{y_0w_x}{y_0w_x}$.

PROGENY NUMBER	PARENTAL COMBINATIONS		NEW COMBINATIONS		TOTAL	CROSSOVERS	CROSSOVER PERCENTAGE
	Y_0W_x	y_0w_x	Y_0w_x	y_0W_x			
4838	10	8	6	3	27	9	33.3
4861	35	36	29	28	128	57	44.5
4863	64	52	48	42	206	90	43.7
4866	31	28	19	21	99	40	40.4
4869	44	42	26	37	149	63	42.3
4870	58	78	46	50	232	96	41.4
4871	32	42	35	31	140	66	47.1
4873	65	67	49	40	221	89	40.3
4876	31	28	15	20	94	35	37.2
4877	27	31	24	17	99	41	41.4
Total	397	412	297	289	1395	586	42.0
Total observed	809		586				
Calculated 1:1	*697.5*		*697.5*				
Difference	111.5 ± 12.6						

TABLE 14

Data on progenies of the backcross $\frac{y_0W_x}{Y_0w_x} \times \frac{y_0w_x}{y_0w_x}$.

PROGENY NUMBER	PARENTAL COMBINATIONS		NEW COMBINATIONS		TOTAL	CROSSOVERS	CROSSOVER PERCENTAGE
	Y_0w_x	y_0W_x	Y_0W_x	y_0w_x			
4839	30	25	15	9	79	24	30.4
4852	54	46	28	39	167	67	40.1
4855	28	25	24	23	100	47	47.0
4874	24	24	11	9	68	20	29.5
Total	136	120	78	80	414	158	38.2
Total observed	256		158				
Calculated 1:1	*207*		*207*				
Difference	49 ± 6.86						

TABLE 15

Data on progenies of the cross $\frac{y_0W_x}{Y_0w_x} \times \frac{y_0W_x}{y_0w_x}$.

PROGENY NUMBER	Y_0W_x	Y_0w_x	y_0W_x	y_0w_x	TOTAL	CROSSOVERS[1]	CROSSOVER PERCENTAGE
4234	24	17	33	7	81	28	34.6
4235	15	6	21	3	45	12	26.7
4293	39	16	34	6	95	24	25.3
4316	21	7	37	6	71	24	33.8
4318	25	8	28	10	71	40	56.3
4319	42	12	37	15	106	60	56.6
4321	54	13	66	14	147	56	38.1
4324	42	11	44	12	109	48	44.0
4326	39	14	32	15	100	60	60.0
4328	38	18	41	11	108	44	40.7
4330	36	8	29	11	84	44	52.4
4332	36	17	51	7	111	28	25.2
4333	25	8	31	6	70	24	34.3
4334	25	6	31	7	69	28	40.6
Total	461	161	515	130	1267	520	41.0

[1] Computed by multiplying the y_0w_x class by 4. See text.

TABLE 16

Data on progenies of the backcross $\frac{y_0CS_h}{Y_0cS_h} \times \frac{y_0cS_h}{y_0cS_h}$.

PROGENY NUMBER	NON-CROSSOVERS		SINGLE CROSSOVERS				DOUBLE CROSSOVERS		TOTAL
			Y_0–C region		C–S_h region				
	Y_0cs_h	y_0CS_h	Y_0CS_h	y_0cs_h	Y_0cS_h	y_0Cs_h	Y_0Cs_h	y_0cS_h	
4234	34	34	6	5	1	1	0	0	81
4235	17	20	3	2	0	2	1	0	45
Total	51	54	9	7	1	3	1	0	126

TABLE 17

Data on progenies of the cross $\frac{y_0 S_A W_z}{Y_0 s_A w_z} \times \frac{y_0 s_A W_z}{y_0 s_A w_z}$.

PROGENY NUMBER	$Y_0 S_A W_z$ 1	$Y_0 S_A w_z$ 2	$Y_0 s_A W_z$ 3	$Y_0 s_A w_z$ 4	$y_0 S_A W_z$ 5	$y_0 S_A w_z$ 6	$y_0 s_A W_z$ 7	$y_0 s_A w_z$ 8	TOTAL 9	SINGLE CROSSOVERS: Y_0–S_A Region[1] 10	SINGLE CROSSOVERS: S_A–W_z Region[2] 11	DOUBLE CROSSOVERS[3] 12
4234	7	0	17	17	30	4	3	3	81	13	16	0
4235	3	0	12	6	20	0	1	3	45	7	0	0
4293	14	0	25	16	33	2	1	4	95	19	8	0
4316	12	1	9	6	32	2	5	4	71	21	8	4
4318	12	1	13	7	27	6	1	4	71	17	24	4
4319	14	0	28	12	34	9	3	6	106	23	36	0
4321	22	1	32	12	56	10	10	4	147	36	40	4
4324	16	0	26	11	40	8	4	4	109	24	32	0
4326	10	1	29	13	29	10	3	5	100	18	40	4
4328	14	1	24	17	35	4	6	7	108	27	16	4
4330	16	0	20	8	28	7	1	4	84	21	28	0
4332	23	1	13	16	47	4	4	3	111	30	16	4
4333	10	1	15	7	29	3	2	3	70	15	12	4
4334	12	1	13	5	31	6	0	1	69	13	24	4
Total	185	8	276	153	471	75	44	55	1267	284	300	32

[1] Sum of the figures in columns 1, 7 and 8. (Column 7 may also contain a few double crossovers)
[2] Four times the figures in column 6.
[3] Four times the figures in column 2.

TABLE 18

Data on F_2 progenies from plants of the constitution $\frac{y_0 C S_A W_z}{Y_0 c s_A w_z}$

PROGENY NUMBER	$Y_0 C S_A W_z$	$Y_0 C S_A w_z$	$Y_0 C s_A W_z$	$Y_0 C s_A w_z$	$Y_0 c S_A W_z$	$Y_0 c S_A w_z$	$Y_0 c s_A W_z$	$Y_0 c s_A w_z$	$y_0 C S_A W_z$	$y_0 C S_A w_z$	$y_0 C s_A W_z$	$y_0 C s_A w_z$	$y_0 c S_A W_z$	$y_0 c S_A w_z$	$y_0 c s_A W_z$	$y_0 c s_A w_z$	TOTAL
4239	116	18	0	0	2	0	22	35	64	10	0	3	0	0	0	3	273
4263	89	17	0	0	3	0	7	9	37	2	0	1	0	0	1	0	166
4295	93	18	0	1	4	0	15	30	50	8	0	1	0	0	0	2	222
4311	28	6	1	1	1	0	3	3	14	1	0	0	0	0	0	0	58
4315	60	13	0	0	0	0	6	12	25	4	0	1	0	0	1	0	122
Total	386	72	1	2	10	0	53	89	190	25	0	6	0	0	2	5	841
Corrected[1]	*386*	*72*	*1.4*	*2.7*	*10*	*0*	*72.1*	*121*	*190*	*25*	*0*	*8.2*	*0*	*0*	*2.7*	*6.8*	*897.9*

[1] The S_A seeds germinated 89.87 percent while the s_A seeds germinated only 66.11 percent. The totals for each of the s_A classes were multiplied therefore by 1.36.

TABLE 19

Data from table 18 arranged to facilitate the calculation of the crossing-over percentages between the various factors (corrected totals used for this calculation).

FACTORS STUDIED	NUMBER OF INDIVIDUALS OF THE KIND STATED				CROSSOVER PERCENTAGE
	Y_0C	Y_0c	y_0C	y_0c	
y_0 and c	462.1	203.1	223.2	9.5	22.4
	Y_0S_h	Y_0s_h	y_0S_h	y_0s_h	
y_0 and s_h	468	197.2	215	17.7	28.6
	Y_0W_x	Y_0w_x	y_0W_x	y_0w_x	
y_0 and w_x	469.5	195.7	192.7	40	36.7
	CS_h	Cs_h	cS_h	cs_h	
c and s_h	673	12.3	10	202.6	2.5
	CW_x	Cw_x	cW_x	cw_x	
c and w_x	577.4	107.9	84.8	127.8	24.4
	S_hW_x	S_hw_x	s_hW_x	s_hw_x	
s_h and w_x	586	97	76.2	138.7	21.6

TABLE 20

Data on F_2 progenies from plants of the constitution $\dfrac{y_0CS_hW_xr}{Y_0cs_hw_xR}$.

PROGENY NUMBER	PURPLE ALEURONE								COLORLESS ALEURONE								TOTAL
	$Y_0S_hW_x$	$Y_0S_hw_x$	$Y_0s_hW_x$	$Y_0s_hw_x$	$y_0S_hW_x$	$y_0S_hw_x$	$y_0s_hW_x$	$y_0s_hw_x$	$Y_0S_hW_x$	$Y_0S_hw_x$	$Y_0s_hW_x$	$Y_0s_hw_x$	$y_0S_hW_x$	$y_0S_hw_x$	$y_0s_hW_x$	$y_0s_hw_x$	
4248	57	10	0	1	33	2	0	0	28	1	5	14	8	0	1	0	160
4300	22	2	0	1	12	0	0	0	8	0	1	0	3	1	0	1	51
4301	62	7	0	0	38	9	0	0	30	4	9	17	13	0	0	0	189
4314	17	1	0	0	5	0	0	0	10	1	1	3	2	0	0	2	42
Total	158	20	0	2	88	11	0	0	76	6	16	34	26	1	1	3	442
Corrected[1]	*158*	*20*	*0*	*3.4*	*88*	*11*	*0*	*0*	*76*	*6*	*27.5*	*58.5*	*26*	*1*	*1.7*	*5.2*	*482.3*

[1] The S_h seeds germinated 86.17 percent while the s_h seeds germinated only 50.0 percent. The totals of each of the s_h classes were multiplied therefore by 1.72.

TABLE 21

Data from table 20 arranged to facilitate the calculation of the crossing-over percentages between the various factors (corrected totals used for this calculation).

FACTORS STUDIED	NUMBER OF INDIVIDUALS OF THE KIND STATED				CROSSOVER PERCENTAGE
	Purple aleurone		*Colorless aleurone*		
	V_g	v_g	V_g	v_g	
y_g and c	181.4	99	168	33.9	17.3
	$V_g s_h$	$V_g s_h$	$y_g S_h$	$y_g s_h$	
y_g and s_h	260	89.4	126	6.9	32.7
	$V_g W_z$	$V_g w_z$	$y_g W_z$	$y_g w_z$	
y_g and w_z	261.5	87.9	115.7	17.2	39.5
	Purple aleurone		*Colorless aleurone*		
	S_h	s_h	S_h	s_h	
c and s_h	277	3.4	109	92.9	9.6
	Purple aleurone		*Colorless aleurone*		
	W_z	w_z	W_z	w_z	
c and w_z	246	34.4	131.2	70.7	26.6
	$S_h W_z$	$S_h w_z$	$s_h W_z$	$s_h w_z$	
s_h and w_z	348	38	29.2	67.1	15.1

TABLE 22

Data on F_2 progenies from plants of the constitution $\frac{y_g S_h W_z}{Y_g s_h w_z}$.

PROGENY NUMBER	$Y_g S_h W_z$	$Y_g S_h w_z$	$Y_g s_h W_z$	$Y_g s_h w_z$	$y_g S_h W_z$	$y_g S_h w_z$	$y_g s_h W_z$	$y_g s_h w_z$	TOTAL
4265	84	6	5	6	28	3	0	0	132
4266	61	9	13	14	25	5	1	3	131
4268	14	0	1	0	6	0	0	0	21
4269	20	0	2	7	8	2	0	0	39
4272	25	3	0	5	8	4	0	0	45
4274	52	5	4	5	24	7	0	0	97
4275	54	9	10	15	28	2	0	2	120
4276	81	8	10	21	27	4	0	2	153
4277	97	11	16	21	43	7	0	2	197
4279	41	4	1	10	21	1	1	1	80
4280	58	6	4	11	27	5	1	3	115
4281	87	4	6	17	50	7	0	1	172
4282	71	3	8	23	38	8	0	5	156
4284	31	2	4	8	15	1	0	0	61
4285	88	9	13	17	45	3	0	1	176
4286	111	11	9	13	60	8	0	3	215
4288	29	3	0	0	13	1	0	0	46
4289	80	10	3	5	35	6	0	0	139
4290	74	9	11	17	27	2	1	3	144
4291	106	9	7	15	33	4	0	1	175
4317	71	9	8	13	22	1	0	1	125
4320	65	9	6	9	41	7	0	2	139
4322	79	4	7	11	43	2	0	1	147
4323	80	9	9	11	40	6	0	2	157
4327	13	0	1	1	2	0	0	0	17
4331	118	13	10	21	62	4	0	2	230
Total	1690	165	168	296	771	100	4	35	3229
Corrected[1]	*1690*	*165*	*278.9*	*491.4*	*771*	*100*	*6.6*	*58.1*	*3561*

[1] The S_h seeds germinated 88.1 percent while the s_h germinated only 53.1. The total of each of the s_h classes were multiplied therefore by 1.66.

TABLE 23

Data from table 22 arranged to facilitate the calculation of the crossing-over percentage between the various factors (corrected totals used for this calculation).

FACTORS STUDIED	NUMBER OF INDIVIDUALS OF THE KIND STATED				CROSSOVER PERCENTAGE
	$Y_g S_h$	$Y_g s_h$	$y_g S_h$	$y_g s_h$	
y_g and s_h	1855	770.3	871	64.7	28.0
	$Y_g W_z$	$Y_g w_z$	$y_g W_z$	$y_g w_z$	
y_g and w_z	1968.9	656.4	777.6	158.1	44.1
	$S_h W_z$	$S_h w_z$	$s_h W_z$	$s_h w_z$	
s_h and w_z	2461	265	285.5	549.5	16.9

INHERITANCE STUDIES IN SOYBEANS. II. GLABROUSNESS, COLOR OF PUBESCENCE, TIME OF MATURITY, AND LINKAGE RELATIONS[1]

F. V. OWEN

Maine Agricultural Experiment Station, Orono, Maine

Received February 18, 1927

TABLE OF CONTENTS

	PAGE
INTRODUCTION	519
Relation of glabrousness to growth and vigor	520
Black eyebrow × glabrous selection No. J5	520
Linkage between P_1 and R_1	521
A single factor for time of maturity	524
Cross between green (No. C4) and glabrous (No. J1)	526
SUMMARY	529
LITERATURE CITED	528

INTRODUCTION

Through the courtesy of Professor ISABURO NAGAI the writer obtained a glabrous mutant variety of soybeans (*Soja max*) the origin and description of which has been reported (NAGAI and SAITO 1923). This variety was given the arbitrary number J 1 in our records. Another glabrous variety from Japan was also furnished by W. J. MORSE of the UNITED STATES DEPARTMENT OF AGRICULTURE and given the arbitrary number J 5.

Many crosses were attempted with these glabrous varieties because the linkage relationships seemed particularly interesting. Due to difficulties in the mechanics of artificial hybridization only a few crosses were successful but the breeding results show much of interest. The F_2 progenies were started in cold-frames in March 1925 and later transplanted in order that they might reach maturity. A limited number of F_3 progenies were also grown at the Arlington Farm during the 1926 season through the courtesy of W. J. MORSE. It would be very desirable to grow more F_3 progenies but it is unlikely that this can be done at the present time.

The crosses to be discussed are as follows:

Black Eyebrow ($p_1R_1TEI^k$) ♀ × Glabrous No. J5 ($P_1r_1teI^i$) ♂

Green No. C4 ($p_1mR_1LI^i$) ♀ × No. J1 (P_1Mr_1li) ♂

[1] From the Department of Genetics, paper No. 73 and Department of Agronomy, WISCONSIN AGRICULTURAL EXPERIMENT STATION. Published with the approval of the Director of the Station.

RELATION OF GLABROUSNESS TO GROWTH AND VIGOR

The dominant factor P_1 as described by NAGAI and SAITO (1923) explains the behavior of the glabrous character in the No. J5 selection as well as in the No. J1 selection obtained from Professor NAGAI. The character is fully dominant in each case but the use of the term "glabrousness" may be somewhat misleading because there are short bristles on the stems and petioles of glabrous plants.

NAGAI and SAITO (1923) also called attention to a decrease in vigor of glabrous plants and the writer has observed the same phenomenon. Glabrous plants grow much more slowly than pubescent plants and are inclined to send out more side branches. The stems are also very woody and tough as compared with those of pubescent plants.

A very similar type of glabrousness to that studied by NAGAI and SAITO (1923) has been reported by STEWART and WENTZ (1926), but instead of being inherited as a dominant character it is strictly recessive. In this case a factor p_2 for glabrousness is assumed to be recessive to a factor P_2 for pubescence. The relation between P_2 and P_1 is naturally of interest because their effects seem to be exactly opposite. Crosses between the two glabrous types would be very desirable to study this relationship.

Another peculiar behavior of the crosses with glabrous varieties has been the occasional occurrence of abnormal plants. In all F_2 populations there has been a close approach to a 3:1 ratio of glabrousness to pubescence, respectively, but a small number were intermediate (4 out of a total of 442 F_2's grown in 1925). The growth of these intermediates was very much reduced, even more than that of the fully glabrous plants. The leaves were somewhat glabrous but the pods were as fully pubescent as those of pubescent plants. These appeared to be abnormalities that cannot be explained by genetic factors. An abnormal chromosome number seems more likely, so the four abnormal plants are not included in the genetic analyses which follow.

BLACK EYEBROW × GLABROUS SELECTION NO. J5

In the F_2 progeny numbers 10, 11, and 80 from crosses between Black Eyebrow and glabrous (No. J5), 251 plants were grown which were normal for either glabrousness or pubescence. The parental varieties in this combination differed in the following pairs of factors:

Black Eyebrow	*Glabrous (No. J5)*
Pubescent, p_1	Glabrous, P_1
Black seed-coat pigment, R_1	Brown seed-coat pigment, r_1
Eyebrow pattern, I^k	Pigment restricted to hilum, I^i
Tawny pubescence, T	Gray pubescence, t (potentially present)
Early maturing, E	Late maturing, e

Factors I^k, I^i and T will be more fully discussed in Part III of this series but brief mention should be made of the effect of T to clarify the classification in table 2. This factor (T) is known to intensify the pigment in the seed-coat as well as in the pubescence. In the F_2 populations, therefore, "imperfect black" and "buff" (see NAGAI 1921) in gray-pubescent plants correspond to black and brown respectively in tawny-pubescent plants, and indicate the presence and absence respectively of the factor R_1.

TABLE 1

The single pairs of factors in progenies 10, 11 and 80 considered separately.

	OBSERVED NUMBERS	DEV	P. E.	$\frac{\text{DEV}}{\text{P. E.}}$	ACTUAL RATIO
Black versus Brown	183: 68	5.25	4.63	1.1	2.7:1
Glabrous versus Pubescent	182: 69	6.25	4.63	1.3	2.6:1
Mottled versus Eyebrow Pattern	186: 65	2.25	4.63	0.5	2.9:1
Tawny versus Gray	169: 64	5.75	4.46	1.3	2.6:1
Early Maturity versus Late Maturity	144:107	44.25	4.63	9.56	1.3:1

In the F_2 generation the presence of T was evident in the field only in the case of the pubescent plants because the hairs on the glabrous plants were too much reduced in development to reveal the desired information. Fortunately this was not a handicap after the plants were ripe because the intensity of the seed-coat color has proved to be a very good index of the presence of T. The classification was therefore completed at that time and breeding results of 12 F_3 progenies have proved the correctness of the classification for a few cases which were somewhat doubtful. It will be observed, however, that no attempt was made to classify the glabrous plants with a brown "eyebrow" pattern for the intensity of color. This was advisable because of the gradations of colors in this particular class. In this case, therefore, the gray-pubescent class is included with the tawny.

The factors I^k and I^i belong to an allelomorphic series, in which i is the recessive for self-color and I^k is dominant for the restriction of all pigment in the seed coat; I^i restricts pigment to the hilum and I^k produces a saddle effect which has been designated the "eyebrow" pattern.

Linkage between P_1 and R_1

Table 1 gives the ratios obtained when each character is considered separately and table 2 gives the combined results from these crosses. Table 2 shows that there is evidently a linkage between P_1 and R_1 that

may be complete, but because of the fact that the progeny segregated for tawny and gray pubescence, the situation is complicated. In the tawny-pubescent class there was no indication of the recessive brown color, but

TABLE 2

F_2 progenies 10, 11, and 80 combined, Black Eyebrow ($p_1R_1I^kT$) ♀ × No. J5 ($P_1r_1I^ii$) ♂.

	GLABROUS PLANTS							
	Tawny pubescence				Gray pubescence			
	Yellow mottled		Eyebrow pattern		Yellow mottled		Eyebrow pattern	
	Black	Brown	Black	Brown	Black	Brown	Black	Brown
Phenotypic formula.....	$P_1R_1I^iT$	$P_1r_1I^iT$	$P_1R_1I^kT$	$P_1r_1I^kT$	$P_1R_1I^ii$	$P_1r_1I^ii$	$P_1R_1I^ki$	$P_1r_1I^ki$
Plants flowering before July 20....	48	26	14	13	5	0	1	..
Plants flowering after July 20....	23	9	3	5	16	15	4	..
Total observed ..	71	35	17	18	21	15	5	
Calculated	*70.6*	*35.3*	*23.5*	*15.7*	*23.5*	*11.8*	*7.8*	..[1]
Deviation	+.4	−.3	−6.5	+2.3	−2.5	+3.2	−2.8	

	PUBESCENT PLANTS							
Phenotypic formula...	$p_1R_1I^iT$	$p_1r_1I^iT$	$p_1R_1I^kT$	$p_1r_1I^kT$	$p_1R_1I^ii$	$p_1r_1I^ii$	$p_1R_1I^ki$	$p_1r_1I^ki$
Plants flowering before July 20....	23	..	12	..	1	..	1	..
Plants flowering after July 20....	5	..	6	..	15	..	6	..
Total observed ..	28	..	18	..	16	..	7	..
Calculated	*35.3*	..	*11.8*	..	*11.8*	..	*3.9*	..
Deviation	−7.3		+6.2		+4.2		+3.1	

$\chi^2 = 13.005 \quad P = .22$

[1] This class was very likely represented but because of difficulties in classification it was included with the tawny pubescent class under the same seed coat pattern. Without this discrepancy the theoretical ratio would have been 18:9:6:3:6:3:2:1:9:3:3:1, but with the system used the ratio becomes 18:9:6:4:6:3:2:0:9:3:3:1.

in the gray-pubescent class the distinction was not always clear. Seven plants from the total of 16 in the "yellow mottled, gray pubescent" class (table 2) were apparently buff rather than imperfect black, as classified in the table, but the writer has had considerable difficulty in distinguishing buffs from imperfect blacks in these crosses. However, breeding behavior in F_3 progenies revealed the fact that these plants really had a factor for the "imperfect black" coloration in the seed coat and that the F_2 plants were homozygous for that factor.

Fortunately there is a very convenient method of growing F_3 progenies to determine the relative amount of crossing over between P_1 and R_1. The glabrous character is easily recognized as soon as the seeds are germinated and a germination chamber suffices for this work. If the linkage were complete between P_1 and R_1 the genotypes $P_1r_1p_1R_1$, $P_1r_1P_1r_1$ and $p_1R_1p_1R_1$ should represent all the possible combinations in the F_2 progeny, that is, all glabrous plants with brown seed-coat color should be homozygous for P_1 and all glabrous plants with black seed-coat color should be heterozygous for P_1. To test this hypothesis F_3 progenies were germinated, giving the following results:

Phenotype in F_2	P_1R_1	P_1r_1
Number of progenies homozygous for P_1	3	15
Number of progenies heterozygous for P_1	20	2

These figures show that the linkage was not complete because 5 progenies from the total of 40 belong to crossover classes which indicates about 12 percent crossing over. This estimate, however, does not include plants that may have been of genotypic constitution $P_1R_1P_1R_1$, which represents a crossover class but which could not be recognized at the time of germination because R_1 has its effect on the seed-coat pigments. One progeny from a total of 10 which were grown to maturity in 1926 from the F_2 phenotype P_1R_1 proved to be of this constitution, that is, it was homozygous for R_1.

The evidence from the breeding behavior in F_3, therefore, indicates that crossing over between P_1 and R_1 is of rather frequent occurrence. These results were somewhat surprising because according to the seed-coat colors of the 46 tawny-pubescent plants in the F_2 generation there was no indication of crossing over. This inconsistency seems very odd but larger numbers must be grown before one can ascribe any reason other than chance. It shows very clearly, however, that a small percentage of crossing over has really taken place.

A single factor for time of maturity

The Black Eyebrow variety matures much earlier than the glabrous paternal parent. The differences and the comparison with the F_1's and F_2's were approximately as follows:

	Date planted	*Time of flowering*	*Time of maturity*
Black Eyebrow	March 13	July 10	Sept. 15
Glabrous (No. J5)	"	Aug. 3	Oct. 15
F_1's	"	July 20	Oct. 5
F_2's	"	(July 5–Aug. 10)	Sept. 10–Oct. 20)

An attempt was made in the F_2 population to make a careful record of the time of flowering but this was done only at certain periods so an exact quantitative record is not available for each plant. The only available method, therefore, was to classify the progeny according to general classes and, to simplify the results, a single division seems advisable. An arbitrary date was set at July 20 to distinguish early-flowering plants from late-flowering plants. At this time the distinction seemed clearer than at any other time because many of the early-maturing plants were setting pods but there were no signs of flowering on most of the late-maturing plants.

There was not a clear-cut 3:1 ratio for time of maturity because the expression of this character was rather variable and a large number of factors for the difference in the parental varieties might as well have been assumed as one, were it not for the high correlation that was found to exist between late maturity and gray pubescence. Table 2 shows this correlation.

If there were no correlation between time of maturity and color of pubescence there should be a simple 3:1 ratio for tawny- and gray-pubescent plants in both the early- and late-maturing classes. These theoretical results in comparison with the observed figures are as follows:

	Early-maturing		*Late-maturing*	
	Tawny	*Gray*	*Tawny*	*Gray*
Observed	123	8	46	56
Calculated, assuming no correlation between T and time of maturity	*98.2*	*32.8*	*76.5*	*25.5*

Applying the χ^2 test, $\chi^2 = 75.6$. This shows that there is a very significant correlation between the time of maturity and color of pubescence.

The data are so much out of agreement with Mendelian expectation that a calculation of crossing over means very little but COLLINS's (1924) modification of YULE's method gives 18 percent crossing over between *T*

and *E*. By comparing the observed and theoretical figures on this basis the following results are obtained.

	Tawny-pubescent		*Gray-pubescent*	
	Early	*Late*	*Early*	*Late*
Observed	123	46	8	56
Calculated, On basis of 18 percent crossing over	*155.2*	*19.5*	*19.5*	*38.8*

The goodness of fit is obviously very poor but there are reasons to believe that it would be better to consider the gray-pubescent class alone. Since heterozygous plants are intermediate for time of maturity, it would be natural, even with complete linkage between *T* and *E*, for a considerable proportion of the heterozygous tawny-pubescent F_2 plants to intergrade into the late-maturing class. Calculating on the basis of the gray-pubescent class alone gives a crossover value of approximately 6 percent. This seems a much more logical value.

It should also be mentioned that 4 of the 8 early-maturing gray-pubescent plants were on the border line in the classification for time of flowering. It is possible, therefore, that they may not have been the result of crossing over. The other 4 plants in this class, however, were among the earliest in the progeny to mature, so there seems to be no doubt that these plants were a result of crossing over between the factors *T* and *E*.

A detailed study of the correlation between time of maturity and color of pubescence in this cross would be very desirable in connection with physiological studies as well as genetic. Since the effect of the relative length of day and night on the time of maturity has been so intensively studied in soybeans, it may be of interest to know the effect of a single Mendelian factor.

Further investigations are necessary to accurately determine the percent of crossing over between *E*, a factor for early maturity, and *T*, a factor for tawny pubescence, but there is little doubt that the factor *E* really exists. This knowledge has been made possible by the linkage between a rather indefinite quantitative character and a well known qualitative character. Examples of a very similar nature in Pisum are summarized in WELLENSIEK's (1925) excellent monograph. The work of LOCK, TSCHERMAK and HOSHINO is particularly interesting in showing the correlation between time of flowering and flower color.

These examples of correlation between time of maturity and qualitative factors are very similar to cases where linkages have been reported with size factors (SAX, 1924 and LINDSTROM, 1926). The desirability of in-

vestigating such linkages cannot be overemphasized because of the application of Mendelian analyses to quantitative characters. By means of linkage relations it is possible to study the effect of a single factor where the data would otherwise result in nothing but confusion.

CROSS BETWEEN GREEN (NO. C4) AND GLABROUS (NO. J1)

The contrasted factors in progenies 91 and 101 are indicated by the following differences in the parental varieties.

Green (No. C4)	*Glabrous (No. J1)*
Pubescent, p_1	Glabrous, P_1
Black pod color, L (Woodworth, 1923)	Yellowish-brown pod color, l
Black seed-coat color, R_1	Brown seed-coat color, r_1
Pigment restricted to hilum, I^i	Self-color, i
Absence of mottling factor, m	Mottling factor, M (Black mottling on brown background)
Green plastid color in seed-coat, G	Yellow plastid color in seed-coat, g
Green cotyledon color, d_1d_2	Yellow cotyledon color, D_1D_2

In these crosses the inheritance of glabrousness was dominant and probably due to the same factor P_1 as in the foregoing crosses with the other glabrous variety (selection No. J5). This gives added interest to the linkage relationship with R_1.

The factor for blackish-brown pod color has been described by WOODWORTH (1923); D_1, D_2 and G are described in Part I of this series; and the factors I^i and R_1 have been discussed in connection with the preceding crosses (Nos. 10, 11 and 80). NAGAI and SAITO (1923) have described the factor M and report a linkage with P_1. About 18 percent crossing over is reported.

Table 3 gives the detailed behavior of this cross for progeny 91 but the cotyledon color and plastid color in the seed coat may be neglected at this time. The main point of interest is the linkage between P_1 and R_1 which has apparently been complete. Table 4 shows this relationship.

Progeny 101 was grown in 1926 at Arlington Farm but the detailed classification given in table 3 for progeny 91 was not made. Table 4, however, shows the relationship between P_1 and R_1 for both progenies.

This evidence indicates complete linkage between P_1 and R_1 but when F_3 progenies were tested in the germination chambers as previously described one case of crossing over was brought to light. Only 5 progenies from glabrous plants were tested but the single crossover shows that P_1 and R_1 were not completely linked. It is logical to suppose, therefore, that the same factor P_1 has been responsible for glabrousness in both of the

TABLE 3

F_2 progeny from Green (No. C4)×Glabrous (No. J1).

	GLABROUS							PUBESCENT		
	Black			*Brown*				*Black*		
	Pigment restricted		Self-Color	Pigment restricted		Self-color		Pigment restricted		Self-color
	Green	Yellow		Green	Yellow	Black mottled	Non-mottled	Green	Yellow	
All yellow cotyledon	16	6	8	1	3[1]	3	1	4	1	2
15 yellow : 1 green cotyledon.....	4		2	2		1		3		
3 yellow : 1 green cotyledon.....	8		5	2				5		2
All green cotyledon.	1					1				
Total	29	6	15	5	3	5	1	12	1	4

[1] Two with black mottling visible.

TABLE 4

F_2 progenies Green No. C4 ($p_1R_1I^c$)×Glabrous selection No. J1 (P_1r_1i).

		GLABROUS				PUBESCENT			
	Progeny Nos.	*Mottled*		*Self-colored*		*Mottled*		*Self-colored*	
		Black	Brown	Black	Brown	Black	Brown	Black	Brown
Observed.....	91	35	15	8	6	13	..	4	..
	101	44	16	10	5	31	..	9	..
Total.....		79	31	18	11	44	..	13	..
Calculated for independent assortment.....		*82.7*	*27.5*	*27.5*	*9.2*	*27.5*	*9.2*	*9.2*	*3.1*
Deviation		−3.7	+3.5	−9.5	+1.8	+16.5	−9.2	+3.8	−3.1
		$\chi^2=2$	8.01	$P=0$	.0002				
Calculated for complete linkage between (P_1 and R_1) .		*73.5*	*36.7*	*24.5*	*12.3*	*36.7*		*12.3*	
Deviation.....		+5.5	−5.7	−6.5	−1.3	+7.3		+.7	

$\chi^2=4.65$ $P=0.46$

Japanese varieties (Nos. J1 and J5) because this factor has been identified in the same linkage group in both varieties.

The mottled pattern on the brown self-colors showed up as expected. Five of the 6 self-browns (table 3) were mottled and 2 plants badly mottled with brown also showed the presence of the black mottling factor. This indicates that the restriction factor I^i has no effect on the expression of M, providing enough brown mottling is produced. Although M and R_1 are both concerned in the production of anthocyanin pigment (M being responsible for concentric rings and R_1 for self-color) in the seed coat, they appear to be separately inherited; but from NAGAI and SAITO'S (1923) results one would assume that they were situated on the same chromosome.

TABLE 5

F_2 progenies, Manchu No. 59 ($p_1R_1I^i$)×Glabrous selection No. J1 (P_1r_1i).

	Progeny Nos.	GLABROUS				PUBESCENT			
		Mottled		*Self-colored*		*Mottled*		*Self-colored*	
		Black	Brown	Black	Brown	Black	Brown	Black	Brown
Observed	83	37	21	17	5	20	1	6	2
	88	27	9	1	2	6	0	2	0
Total		64	30	18	7	26	1	8	2
Calculated for independent assortment		*65.8*	*21.9*	*21.9*	*7.3*	*21.9*	*7.3*	*7.3*	*2.4*
Deviation		−1.8	+8.1	−3.9	−0.3	+4.1	−6.3	+0.7	−0.4

$\chi^2 = 10.090 \quad P = 0.18$

The results from a very similar cross (Manchu No. 59, $p_1R_1I^i$ ♀ × No. J1 P_1r_1i ♂) are reported in table 5. These results also show a linkage between P_1 and R_1. More numbers would be very desirable to increase the accuracy of the estimate of crossing over but the same factors p_1, R_1, and I^i explain the results very well thus far.

SUMMARY

1. A dominant factor P_1 for glabrousness has been described which is linked with R_1 the factor for black seed-coat color, but from the limited data available an accurate estimate of the amount of crossing over can not be given.

2. Attention is called to a description of another factor p_2 (STEWART and WENTZ, 1923) which produces the same effect as P_1 but is inherited as a recessive rather than as a dominant factor.

3. A factor *E* has been assumed responsible for early maturity in the Black Eyebrow variety because of the peculiar correlation with *T*, the factor for tawny pubescence. Both of these factors, however, are inherited independently of P_1.

LITERATURE CITED

COLLINS, G. N., 1924 Measurement of linkage values. Jour. Agric. Res. **27**: 881–891.

LINDSTROM, E. W., 1926 Hereditary correlation of size and color characters in tomatoes. Iowa Agric. Expt. Sta. Technical Bul. **93**.

NAGAI, I., 1921 A genetico-physiological study on the formation of anthocyanin and brown pigments in plants. Jour. Coll. Agric., Imp. Univ., Tokyo **8**: 1–92.

NAGAI, I., and SAITO, S., 1923 Linked factors in soy-bean. Japan. Jour. Bot. **1**: 121–136.

SAX, KARL, 1924 The nature of size inheritance. Proc. Nat. Acad. Sci. **10**: 224–227.

STEWART, RALPH T., and WENTZ, JOHN B., 1926 A recessive glabrous character in soybeans. Jour. Amer. Soc. Agron. **18**: 997–1009.

WELLENSIEK, S. J., 1925 Genetic monograph on Pisum. Bibliographia Genetica **2**: 343–476.

WOODWORTH, C. M., 1923 Inheritance of growth habit, pod color, and flower color in soybeans Jour. Amer. Soc. Agron. **15**: 482–495.

THE EFFECTS IN COMBINATION OF THE MAJOR COLOR-FACTORS OF THE GUINEA PIG*

SEWALL WRIGHT

Department of Zoology, University of Chicago, Chicago, Illinois

Received April 4, 1927

TABLE OF CONTENTS

	PAGE
INTRODUCTION	530
The color factors of the guinea pig	530
The grades of colors	532
Grades on color wheel	533
Combinations of the albino series with factors *b* and *p*	535
The dilution factor *f*	538
Combination of factors *f* and *b*	543
Combination of factors *f* and *p*	544
Imperfect dominance of *f*	546
Absence of linkage	547
Environmental effects	548
DISCUSSION	552
Relations of factor effects	555
SUMMARY	566
LITERATURE CITED	567

INTRODUCTION

The major color variations which have been reported in the guineapig are due to seven series of allelomorphic factors. The possible combinations among these factors account for 43,740 genetically distinct sorts of guinea pigs. The effects of any given factor can thus be studied on a great variety of genetic backgrounds. The primary purpose of the present paper is to present a survey of the effects of factor differences on the appearance of coat color.

THE COLOR FACTORS OF THE GUINEA PIG

The golden agouti variety of guinea pig is a convenient starting point for consideration of the color varieties, as having the highest dominant factor in all seven of the series of factors, and also as agreeing with the wild Peruvian cavy (*Cavia cutleri* Bennett) in this respect.[1] It has two kinds of pigmentation in its coat, each hair being black with an intense

* The data for this paper were in the main obtained in experiments carried on by the author in the Bureau of Animal Industry, U. S. DEPARTMENT OF AGRICULTURE.

[1] The color of the wild cavy is much paler than that of the golden agouti guinea pig owing to residual heredity independent of the main color factors (WRIGHT 1916).

yellow or "red" (ochraceous tawny) subterminal band. This band is wider and lighter in color on the belly which thus appears nearly or quite self yellow.

The major color factors[2] may be divided into three groups according to their effect on the pattern. In the first group are those factors whose effect is a restriction of the colored areas to spots on a white ground. The factor *s* is the primary factor for bringing out a piebald pattern of this sort. The extent and localization of the white areas is affected by minor genetic factors, age of dam, and sex, but to the greatest extent by nongenetic developmental irregularities of a sort not common even to litter mates (WRIGHT 1920, 1926).

In the second group are those factors which have to do with what seems to be the primary qualitative differentiation of pigment, that of a black or sepia series and a yellow series. With factor *e* in place of *E* (CASTLE 1905), the entire coat is of some color of the yellow series, except for more or less "sootiness" which usually develops with age. The eyes and the skin in exposed parts, however, develop black pigment. There is an intermediate allelomorph, e^p (IBSEN 1916), responsible for a mosaic coat pattern, in which spots of a dark color, like that due to *E*, are found on a ground color of the same sort of yellow as that brought out by *e*. As in the case of the piebald pattern, this "tortoise-shell" pattern is affected by minor genetic factors, sex, and nongenetic developmental irregularities.

Another factor which may be placed in this group is *a* (CASTLE 1905) which in place of *A* causes the pigmentation of the dark areas of the fur to be wholly of the black series instead of agouti. There is an intermediate allelomorph a^r (DETLEFSEN 1914) derived from crosses with the Brazilian cavy (*C. rufescens* Lund) which has an intermediate effect. The animal has an agouti pattern as with *A* but a pattern in which the width of the yellow subterminal band is reduced.

The appearance of guineapigs with the various combinations of factors considered above, omitting a^r, is as follows, assuming that the remaining four series are represented by the dominant allelomorphs (*CFPB*).

[2] The seven series of color factors discussed in this paper (*S, E, A, C, F, P, B*) are the same as those designated by the same symbols in previous papers by the author and agree with CASTLE'S symbolism except in certain of the superscripts used for multiple allelomorphs (cf. CASTLE'S review of the literature in Bibliographia Genetica 1925). The symbolism given in KOSSWIG'S recent review (1925) may be homologized by equating his *S, B, G, A, E* and *C* to *S, E, A, C, P* and *B*, respectively, of the present paper.

	S	*s*
EA	Agouti	Agouti—white
Ea	Black	Black—white
e^pA	Agouti—red	Agouti—red—white
e^pa	Black—red	Black—red—white
eA	Red	Red—white
ea	Red	Red—white

The third group includes four series of factors which modify the quality or intensity of the colors of the black or yellow series without disturbing their distributions as determined by the first two groups of factors.

The effects of the 15 compounds in one of these series (the albino series C, c^k, c^d, c^r, c^a) on black and yellow in the presence of the dominant factors of the other three series (*FPB*) was the subject of a preceding paper (WRIGHT 1925).

The most favorable combination among factors of the first two groups for study of the third is that of the black-red tortoise-shells (Se^pa) since in these the entire coat is colored and it is possible to grade both black and yellow. Agoutis, self blacks and self reds have also been produced in many combinations and have given valuable supplementary information.

THE GRADES OF COLORS

The colors of the guineapigs used in the present study were graded within a week of birth, with the help of three series of skins. As described in the paper referred to above, the sepia series runs from grade 3, a light "sepia," to 14, intense black. The yellow series runs from grade 1, a very pale "cream" barely distinguishable from white, to 13, an intense "red." The "pale sepia" series runs from grade 1, a pale slate color, barely distinguishable from white, to 12, a light sepia, practically identical with grade 5 of the "sepia" series.

The sepia series has been used for grading both sepias (*PB*) and browns (*Pb*). There is a slight difference in quality, but not so great as to make it difficult to use the same set of skins for both. As a matter of fact, the sepia skins have been used as standards only after considerable seasoning, a process in which they have faded somewhat and become slightly browner than at first. The skin has been used for a lower grade than that originally assigned the animal. The final quality is about as close to brown as sepia.

The yellow series has been used for grading the yellow areas of tortoise-shells and for the cream which appears in place of black in the combination

fp. There is, of course, a great qualitative difference between this series and the other two.

The "pale sepia" series has been used for grading the pale sepia and pale brown found in combinations *FpB* and *Fpb*, respectively. The standard series of skins (all *FpB*) grades continuously into the sepia series as noted above. For the purpose of the present paper it will be convenient to combine these into one series running from white (0) to grade 21, intense black, by adding 7 to all grades with the first series of skins. The averages for the albino series, previously published (WRIGHT 1923, 1925) are here modified in this way.

Qualitative differences were most troublesome in grading the classes of animals for which the pale sepia series of skins was used. The grading of the pale browns (*Fpb*) was probably the least satisfactory. As with the sepia series, however, the standard skins had become somewhat browner than at first before being used as standards.

Even among the pale sepias (*FpB*) there was difficulty in comparing all of the albino series compounds. Those with the light dilution factor (c^d*FpB*) were yellowish slate in color even approaching yellow in extreme cases in contrast with the more neutral slate color of combinations of *C*, c^k or c^r with *FpB*. The standards were taken from this latter group, but animals with the combination c^d*FpB* were graded by the same series.

GRADES ON COLOR WHEEL

In spite of the difficulties outlined above, the use of standard skins probably gives the most rapid and reliable means of obtaining the order of intensity of fur colors graded at different times. The color of the standard skins and also of a considerable number of previously graded live animals have been matched on a color wheel (Milton Bradley Co.), as suggested by DAVENPORT (1904), using mixtures of white, yellow, orange and black. The small color wheel was spun directly on the skins to be matched or beside the live animal in a room with diffuse daylight (north illumination only). The proportion of the colors necessary for matching the skins varies somewhat, depending on the illumination, more black and less white becoming necessary as the illumination increases.

Table 1 shows the combination of colors which matched the sepia series after averaging and slightly smoothing the results of a number of determinations. The browns (not pink-eyed) appear to differ from the sepias in having slightly more yellow and orange at a given grade. The difference is very slight, however. The animals themselves have a different appearance from sepias, more because of the difference in the color of the skin

in exposed parts (light brown in place of black) than because of any difference in the color of the fur.

TABLE 1

The proportions of white (W) yellow (Y), orange (O) and black (B) on the Milton Bradley color wheel which match the standard grades of sepia.

GRADE	W	Y	O	B	$\frac{O}{Y+O}$	GRADE	W	Y	O	B	$\frac{O}{Y+O}$
0	85	10	0	5	0	11	6	7	6	81	.46
1	63	11	6	20	.35	12	5	6	5	84	.45
2	50	12	8	30	.40	13	4	5	4	87	.44
3	40	12	8	40	.40	14	3	4	3	90	.43
4	32	12	8	48	.40	15	2	3	3	92	.50
5	26	12	8	54	.40	16	1.4	2.3	2.3	94	.50
6	21	11	8	60	.42	17	1	2	2	95	.50
7	16	11	8	65	.42	18	0.4	1.8	1.8	96	.50
8	13	10	7	70	.41	19	0	1.5	1.5	97	.50
9	10	9	7	74	.44	20	0	1	1	98	.50
10	8	8	6	78	.43	21	0	0.5	0.5	99	.50

Table 2 shows the color combinations which matched the standard yellow series after averaging and smoothing the separate determinations. An interesting feature is the increase in the proportion of orange relative to yellow with rising intensity of color. This change is indicated but much

TABLE 2

The proportions of white (W), yellow (Y), orange (O) and black (B) on the Milton Bradley color wheel which match the standard grades of yellow.

	W	Y	O	B	$\frac{O}{Y+O}$
0	85	10	0	5	0
1	62	24	5	9	.17
2	46	31	9	14	.22
3	34	34	12	20	.26
4	25	34	15	26	.31
5	18	32	18	32	.36
6	13	29	20	38	.41
7	10	25	21	44	.46
8	7	21	22	50	.51
9	5	17	21	57	.55
10	3	14	20	63	.59
11	2	12	17	69	.59
12	2	10	14	74	.58
13	2	8	11	79	.58

exaggerated in the popular terms "yellow" and "red." All grades are in fact intermediate between yellow and orange.

The nature of the qualitative differences among the colors is shown in figure 1, in which those grades in the various series, which require the same amount of black and orange combined, are compared.

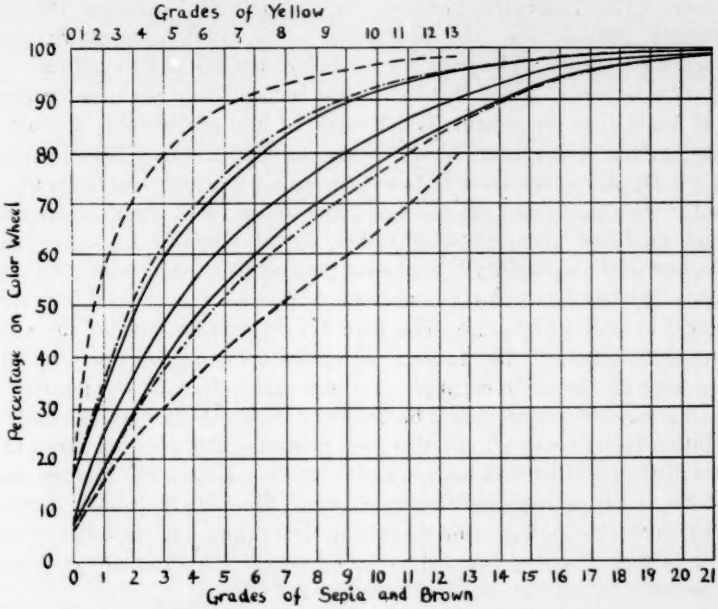


FIGURE 1.—The percentage composition of the standard grades of sepia (solid), brown (dot and dash) and yellow (dash) in terms of black, orange, yellow and white (reading from the bottom) on the Milton Bradley color wheel. The yellow scale is distorted in such a way that the total black and orange agrees with the grades on the sepia (and brown) scale. Note that these scales differ principally in the proportions of neutral gray (black and white) mixed with the spectral hues (yellow and orange).

COMBINATIONS OF THE ALBINO SERIES WITH FACTORS *b* AND *p*

The methods by which the 15 compounds of the albino series were produced and tested (*FPB* present) have been described in some detail in the 1925 paper. It does not seem necessary to go into the details of the matings by which these same 15 compounds were combined with the pink-eyed factor (*p*) (CASTLE 1914) the brown factor (*b*) (CASTLE 1907) and with both combined (*pb*) as the general effects and the simple recessive nature of these factors have long been established.

One set of matings (L) was largely devoted to combining the pink eye factor with compounds of the albino series. The matings here considered

were practically free from factor *b* and wholly free from factor *f*. Another set of matings (N) was similarly devoted to combining the brown factor (*b*) with the albino series and was also wholly free from *f*. A few pink-eyed browns (*ppbb*) appeared, however. In a third set of matings (UR) the primary purpose was the production of a multiple recessive stock ($sse^pe^paac^kc^kppbb$). This was started before the distinction between the dilution factors c^k and c^d had been made but on study of the pedigrees it was found that the dilution factor involved in a considerable number of matings traced back exclusively to sources of c^k and in a few to sources of c^d. Dilutes which trace to both sources are omitted. As factors *C*, c^r and c^a were also present in the foundation stock, all of the 15 compounds were produced although with rather scanty numbers in some cases. Because of the possibility of confusing pink browns (*ppbb*) with pink-eyed sepias (*ppBB*) care was taken that the pink-eyed browns used in mating should be derived by segregation from brown-eyed browns (*PPbb*) which are unmistakable. Throughout experiments L and N (and UR after checking up the early matings) care was taken to make the matings in such a way that there should be no doubt from the ancestry as to which dilution factor (c^k or c^d), if either, was present. This does not mean that two distinct strains were kept in each case since albinos (c^a) and red-eyed dilutes (c^r) from parents carrying either of the dilution factors, c^k or c^d, were freely crossed with animals tracing their dilution to the other source. The factors c^k and c^d themselves were brought together only in crosses especially intended to produce the compound c^kc^d.

In case of matings producing more than one class of young, use was made of the grade of yellow in assigning genetic constitutions. Neither *p* nor *b* have any recognizable effect on grades of yellow in any combination which the writer has made. It has seemed safe from the results described previously (WRIGHT 1925) to assume that pink-eyed or brown animals (factor F present) showing red of grade 9 or more were intense (*C*-) that those showing a yellow of grade 7 or 8 were dilutes of one of the compounds indicated by the symbols $c^{kd}c^{kd}$, that those showing cream of grades 3 to 5 were dilutes of one of the four types included in the symbol $c^{kd}c^{ra}$ and finally that those showing white in a pattern resembling tortoise-shell were either c^rc^r or c^rc^a. There is some overlapping of the above limits but such cases are so uncommon as to introduce no important error. Yellows of grade 6, however, are frequently either $c^{kd}c^{kd}$ or $c^{kd}c^{ra}$. Assignment has been made, if at all, on the basis of contrast with litter mates, where not certain from ancestry or descendants.

By taking into account ancestry, descendants and grade of yellow, nearly all of the animals produced in experiments L and N could be assigned to one of the 15 albino series compounds with reasonable certainty. A number of pink-eyed and brown animals which segregated from the experiments (J and K) discussed in the previous study were also available. It may be added that much use has been made of true breeding

TABLE 3

Distribution of grades of brown in animals of constitutions FFP-bb and different known constitutions relative to the albino series.

GENETIC CONSTITUTION	WHITE	GRADE OF BROWN *FFP-bb*									NUMBER	MEAN	σ
	0	10	11	12	13	14	15	16	17	18			
$C-$					4	12	17	43	15	2	93	15.63	1.11
$c^k c^k$				3	5	40	25	20	1		94	14.61	1.00
$c^k c^d$					1	7	8	8	6		30	15.37	1.13
$c^k c^r$			1	1	1	19	12	5	6		45	14.76	1.27
$c^k c^a$				5	27	37	5	1			75	13.60	0.77
$c^d c^d$				2	15	30	22	5			74	14.18	0.91
$c^d c^r$				1	3	6	16	11	2		39	15.00	1.09
$c^d c^a$		4	3	1	13	5					26	12.46	1.34
$c^r c^r$					1	2	5	4	2	1	15	15.47	1.25
$c^r c^a$			3	7	24	12	2				48	13.06	0.00
$c^a c^a$	X									–	–	0.00	–

TABLE 4

Distribution of grades of pale sepia in animals of constitution FFppB and of different known constitutions relative to the albino series.

GENETIC CONSTITUTION	WHITE	GRADE OF PALE SEPIA *FFppB-*												NUMBER	MEAN	σ
	0	1	2	3	4	5	6	7	8	9	10	11	12			
$C-$							1	1	6	4	22	8	1	43	9.70	1.18
$c^k c^k$							1	5	9	38	16	6		75	9.08	1.02
$c^k c^d$							1	4	4	2				11	7.64	0.87
$c^k c^r$						2	2	2	2	2				10	7.00	1.41
$c^k c^a$						9	5	1						15	5.47	0.61
$c^d c^d$			1	10	30	53	21	7	1					123	4.88	1.04
$c^d c^r$			3	15	12	16	1	1						48	4.00	1.08
$c^d c^a$		5	9	17	4									35	2.57	0.87
$c^r c^r$			12	10	2									24	2.58	0.64
$c^r c^a$	6	7	2											15	0.73	0.68
$c^a c^a$	X													–	0	–

TABLE 5

Distribution of grades of pale brown in animals of constitution FFppbb and of different known constitutions relative to the albino series.

GENETIC CONSTITUTION	WHITE	GRADE OF PALE BROWN *FFppbb*										NUMBER	MEAN	σ
	0	1	2	3	4	5	6	7	8	9	10			
$C-$								1	4	1	1	7	8.29	0.87
$c^k c^k$						5	29	110	64	12		220	7.22	0.83
$c^k c^d$						2	6	2				10	6.00	0.63
$c^k c^r$				1	1	13	32	25	1			73	6.12	0.87
$c^k c^a$						15	10	3				28	5.57	0.68
$c^d c^d$				1		3	1	2				7	5.43	1.29
$c^d c^r$				1	2							3	3.67	0.47
$c^d c^a$				5	1							6	3.17	0.37
$c^r c^r$		2	4	6	5							17	2.82	0.99
$c^r c^a$	4	17	7									28	1.11	0.61
$c^a c^a$	X											–	0.00	–

stocks (included in L, N and UR) of types $c^k c^k ppBB$, $c^k c^k PPbb$, $c^d c^d ppBB$ and $c^d c^d PPbb$ in producing the various compounds. Tables 3, 4 and 5 show the distribution of grades of sepia and brown in the combinations of *pp*, *bb*, and *ppbb*, with the albino series compounds. The averages are brought together in table 17. The significance of the differences in the averages within each group are discussed later.

THE DILUTION FACTOR *f*

In the case of combinations involving factor *f* it is desirable to go into greater detail, since only scanty data have previously been published on this factor (WRIGHT 1923).

All yellows and creams which the writer has tested have proved to owe their dilution of color to an intermediate allelomorph of albinism (c^k or c^d) with the exception of descendants of three animals, two sisters and the very remotely related mate of one of these (matings B205 and B211) in a stock (B) which had been maintained for many years without outcross, as a control in the inbreeding experiments of the Bureau of Animal Industry. The factor was probably present in the stock from the first as many black-yellows and yellow-agoutis were recorded but it was not recognized as new, since factor c^k, with closely similar effects, was also present. In mating B211 an albino male was mated with an intense agouti female. The progeny consisted of 11 intense and 5 dilute. While it seemed probable that the mating was of type $c^a c^a \times Cc^k$, attention was attracted

to it by the grade of intensity of the yellow in the dilute young which was distinctly higher than encountered, except very rarely in dilutes of formula $c^k c^a$. The dilutes from this mating were of grade 7 or 8, while $c^k c^a$ is typically of grade 4 or 5. The black associated with the yellow moreover showed no dilution whatever. Two of the dilute young were mated with each other, producing 6 dilutes (yellow-agouti) and 2 albinos. Four matings were made between these dilutes and albinos of the stock used in studying the effects of the albino series compounds. In previous experiments albino by dilute, regardless of ancestry on either side, had never given intense young. These four matings, however, produced 17 unmistakably intense young (black-red, or golden-agouti), in addition to 5 intense blacks and 26 albinos. This result practically demonstrated that a new recessive dilution factor (*f*) was present which was independent of the albino series. These intense young were backcrossed with albinos from the same stock as in the previous generation. Again, only intense and albinos appeared (9 with red in the fur, 10 intense black and 24 albinos). It is thus clear that albinos can transmit the intense allelomorph of the new dilution factor. As a complement to this experiment, two of the albinos from the first of the above crosses were mated with ordinary dilutes. The young were all ordinary dilutes (2 cream agouti, $c^k c^a$, and 3 medium sepia, $c^d c^a$). The albinos from this source thus do *not* transmit the intense allelomorph of ordinary dilution nor any intense modifier. Similarly, dilutes tracing to mating B211 were mated with ordinary dilutes ($c^k c^k$ and $c^d c^d$). The four matings produced 21 intense (red in fur) and 21 dilute (yellow or cream in fur). The occurrence of intense young proves that each of the two kinds of dilution supplies the dominant intensity factor lacking in the other. The dilute young were expected from matings of type $Cc^{da}ff \times c^{kd}c^{kd}FF$. Four matings of intense from the first cross of *ff* dilutes with ordinary albinos, and thus necessarily Cc^aFf were made with albinos from the same type of mating and thus necessarily $c^a c^a Ff$. The young consisted of 20 intense with red in the fur, 4 dilutes with yellow in the fur, 11 blacks which might belong with either of the foregoing classes and 35 albinos. This result is reasonably in accord with expectation. A mating between two double heterozygotes ($Cc^aFf \times Cc^aFf$) produced 14 intense with red in the fur, 5 dilutes with yellow in the fur, 1 black (intense or dilute) and 10 albinos, also in good agreement with the hypothesis of a dilution factor independent of albinism, but impossible for a dilution factor allelomorphic with albinism.

It was next of interest to find the combined effect of the two series of dilution factors. An intense (black-red) from a mating $c^d c^d FF \times Cc^a ff$

was mated with an intense (black-red) from a mating $Cc^{a}ff \times CCFF$. As ordinary dilutes were produced, this mating was of the type $Cc^{d}Ff \times Cc^{a}Ff$. Black reds (C–F–), black-yellows (C–ff) and a light sepia-cream ($c^{d}c^{a}F$–) were produced in the earlier litters. There appeared some blacks with cream spotting. It was at first thought likely that these were the desired combination $c^{d}c^{a}ff$, but one which was tested by mating with an ordinary dilute ($c^{d}c^{d}FF$) gave intense young, proving that it was C–ff, although with more dilution of yellow than had been encountered up to that time. The effect of factor f has in fact proved to be rather variable, as may be seen from table 6. Meanwhile, this mating gave 3 light or medium sepias, which were brindled with *white*, giving them the appearance of red-eyed dilute tortoise-shells ($c^{r}c^{a}$) except for their black eyes. One of these was tested with an ff dilute ($CCff$). It produced 14 black-yellow or black-cream young (C–ff) proving it to have been of the desired combination. The mating which we have been discussing ($Cc^{d}Ff \times Cc^{a}Ff$) produced altogether 10 black-red (C–F–), 6 black-yellow or black-cream (C–ff), 1 sepia-cream ($c^{d}c^{a}F$–) and 4 sepia-white (3 brindled with white, $c^{d}c^{a}ff$, but 1 with merely piebald white and proved by breeding test to be $c^{d}c^{a}F$–).

A large number of black-eyed sepias brindled with white have since been produced where $c^{d}c^{a}ff$ was expected. Black-eyed silver agoutis (each hair sepia with a white instead of red or yellow band) have also been produced in cases where the agouti factor was present. It thus appears that ff in combination with $c^{d}c^{a}$ reduces cream to white. There have been a few cases, however, in which this reduction was not complete. A white brindled sepia mated with an albino, homozygous in the new dilution factor ($c^{d}c^{a}ff \times c^{a}c^{a}ff$) produced 6 albino young, 6 light sepia-white, 3 silver agoutis of the kind described above, but also 2 light sepias with brindling in which there was clearly a slight creamy tint (grade 1) and 1 silver agouti with a slight creaminess on the nose. The creamy white of these animals was much paler than the cream of ordinary heterozygous dilutes ($c^{d}c^{a}F$), which are seldom graded less than 4. As shown in table 6, 69 out of 74 known to be $c^{d}c^{a}ff$ had white in place of cream, 3 had cream of grade 1, one of grade 2, and one of grade 3. The grade of sepia in these animals (tables 7, 17) was not significantly different from that of $c^{d}c^{d}FF$ (13.9 in place of 14.0). Comparison of $c^{d}c^{a}ff$ and $c^{d}c^{a}F$– from the same litter has revealed no consistent difference. These results suggest that factor f is a diluter of yellow only, a conclusion which, however, it will be seen must be modified in dealing with combination with factor p.

A number of guineapigs of formula $c^{d}c^{d}Ff$ were produced from a mating $c^{d}c^{d}FF \times Cc^{d}ff$. These were indistinguishable from ordinary homozygous

dilutes c^dc^dFF. Two of them were mated with each other to produce the combination c^dc^dff. The young consisted of 11 medium sepia-yellow like c^dc^dF- (sepia grade 14 to 17, yellow grade 6 or 7), and 4 medium sepia-pale cream (sepia grade 15 or 16, cream grade 2 or 3). The assumption that the latter were c^dc^dff attributes a similar effect to ff in this combination, as in c^dc^aff, that is, further dilution of yellow but no further dilution of sepia. As a breeding test two of those believed to be c^dc^dff were mated with an albino, known to be c^ac^aff (a segregate from a mating $Cc^aff \times Cc^aff$). They produced 23 young, all light to medium sepia and with the white or, in a few cases, the creamy white brindling, characteristic of c^dc^aff. The combination c^dc^dff has also been produced from mating $c^dc^aff \times c^dc^aff$. Four such matings produced 6 medium sepia-pale cream (c^dc^dff), 23 light sepia-white (c^dc^aff) and 12 albinos (c^ac^aff). These matings have given a rather curious illustration of the vagaries of dominance. One litter, for example, contained a black-eyed medium sepia (17)—cream (1), a black-eyed light sepia (13)—white (0), and an albino. In this litter, containing the types c^dc^dff, c^dc^aff and c^ac^aff, respectively, there was complete dominance (at least superficially) of the dark eye color of c^d, imperfect dominance of c^d in the sepia parts of the coat and apparently complete dominance of the albino factor c^a in the cream parts. By a suitable combination of factors (eec^dc^affpp) one could produce a strain in which pink-eyed cream would appear as a recessive to albinism, the latter due to a factor c^a, often cited as the type of a recessive factor.

A stock of medium sepia-pale cream (c^dc^dff) has been developed. These do not differ from c^dc^dFF in grade of sepia (16.8 in place of 16.9) but are consistently graded 0 to 3 in the cream parts of the fur in place of 6 or 7. The combination eec^dc^dff has been produced in two animals. One was a self colored black-eyed cream, grade 3, while the other was called black-eyed white. As it was born dead and was disposed of before grading it is possible that it was really a very pale cream.

The combination c^dc^rff has been bred from a number of matings. Thus, 3 matings of type $c^dc^dff \times Cc^rFf$ have produced 5 black-red (Cc^dFf), 4 black-yellow (Cc^dff), 3 dark sepia-cream (c^dc^rFf) and 6 dark sepia with white or pale cream brindling (c^dc^rff). In all of these dilutes the grades of sepia (18 or 19) have been the same as those most characteristic of c^dc^rFF but the cream in the latter has been reduced in c^dc^rff to white or occasionally very pale cream below the limit of c^dc^rFF.

Sixteen red-eyed sepia-whites or silver agoutis have been produced from matings which should produce equal numbers of c^rc^aff and c^rc^aFf. It is reasonably certain that some of these are of the desired combination

$c^r c^a ff$ but this has not yet been proved by an adequate breeding test. From the appearance of these 16 animals and on analogy with the lack of effect of *ff* on the sepia of $c^d c^d$, $c^d c^r$, and $c^d c^a$ it is probable that the combination with red-eyed dilution will have no visible effect whatever, since the red-eye factor by itself reduces yellow to white.

The combinations $c^k c^k ff$ and $c^k c^a ff$ have been made in a few animals, nearly all silver (or very pale cream) agoutis. Here again factor *f* appears to have no effect on intensity of sepia but to reduce yellow ($c^k c^k$) and cream ($c^k c^a$) to very pale cream and white, respectively. The interpretation of the crosses involving c^k and *f* is complicated by the fact that the two types of dilutes $c^k c^k FF$ and $CCff$ are both black-yellows and virtually indistinguishable in appearance.[3] In the former there is undoubtedly a slight tendency to dilution of black, absent in the latter, but the difference is not enough to give a safe criterion for distinguishing animals which may be of either type.

TABLE 6

Distribution of grades of yellow in animals of constitution ff and of different known constitution relative to the albino series.

GENETIC CONSTITUTION	WHITE	GRADE OF YELLOW *ff*									NUMBER	MEAN	σ
		Pale cream		Cream			Yellow			Red			
	0	1	2	3	4	5	6	7	8	9			
$C-$				1	2	20	33	186	18	2	262	6.77	0.76
$c^k c^k$		4	1								5	1.20	0.40
$c^k c^a$	21										21	0.00	0.00
$c^d c^d$	9	34	30	16							89	1.60	0.89
$c^d c^r$	8		1								9	0.22	0.63
$c^d c^a$	69	3	1	1							74	0.11	0.45
$c^r c^r$	1										1	0.00	0.00
$c^r c^a$	X										X	0.00	0.00
$c^a c^a$	X										X	0.00	0.00

The effects of *ff* on the albino series compounds in graded animals is given in tables 6 and 7 with averages in the appropriate columns in tables 17 and 18. The general conclusion already suggested, that *ff* has no visible effect on grades of sepia but greatly reduces yellow pigmentation is

[3] The appearance at birth and up to 2 or 3 months of age is here referred to. Later in life the two varieties may readily be distinguished by the persistence of the yellow color of $c^k c^k FF$, but fading of that of $CCff$ to a cream or even white.

TABLE 7

Distribution of grades of sepia in animals of constitution ffP-B-and of different known constitutions relative to the albino series.

GENETIC CONSTITUTION	WHITE	GRADE OF SEPIA ffP-B-												NUMBER	MEAN	σ
		Light				Medium			Dark			Black				
	0	10	11	12	13	14	15	16	17	18	19	20	21			
$C-$											2	10	139	151	20.91	0.32
c^kc^k											1			1	19.00	..
c^kc^a										2				2	18.00	..
c^dc^d					2	1	6	13	11	15	5			53	16.79	1.45
c^dc^r										4	5			9	18.56	0.50
c^dc^a		1	1	2	18	26	16	2	1					67	13.91	1.12
c^ac^a	X														0.00	0.00

brought out. The red of $C-$ is reduced to a yellow or occasionally a cream which fades in later life to cream or even white. The yellow of c^kc^k or c^dc^d is reduced to a pale cream typically and probably a pure white in exceptional cases. The cream of c^kc^a, c^dc^r, and c^dc^a is reduced typically to pure white, with occasionally a trace of persistent creaminess. The white of albinos and doubtless also of red-eyed dilutes is unchanged.

COMBINATION OF FACTORS *f* AND *b*

A few matings have been made with the purpose of combining *f* with the brown factor, *b*. The latter changes black to brown in fur, skin and eyes, but is without effect on yellow pigment. The only two certain

TABLE 8

The segregation in F_2 following matings of the type $CCffPP \times c^dc^dFFpp$, or of $Cc^dffPP \times c^dc^dFFpp$.

FUR COLOR		EYE COLOR	GENETIC CONSTITUTION	$Cc^dFfPp \times Cc^dFfPp$		$Cc^dFfPp \times c^dc^dFfPp$	
"Black" areas	"Yellow" areas			Observed	Calculated	Observed	Calculated
Black	Red	Black	CFP	55	59.1	18	21.4
Medium sepia	Yellow	"	c^dc^dFP	18	19.7	24	21.4
Black	Yellow	"	$CffP$	23	19.7	11	7.1
Pale sepia	Red	Pink	$CFpp$	22	19.7	7	7.1
Medium Sepia	Pale Cream	Black	c^dc^dffP	9	6.6	7	7.1
Pale Sepia	Yellow	Pink	c^dc^dFpp	8	6.6	5	7.1
Pale Cream	Yellow	"	$Cffpp$	4	6.6	2	2.4
White	Pale Cream	"	c^dc^dffpp	1	2.2	2	2.4
				140	140.2	76	76.0

combinations made are a brown-yellow, *C–ffbb*, and a brown-pale cream, c^dc^dffbb. The brown parts showed ordinary brown grades, 16 and 15, respectively, which are typical of browns *C–FFbb* and c^dc^dFFbb. A number of less certain cases tend to confirm the view indicated by these two cases that factor *f* has no effect on the intensity or quality of brown in albino series compounds.

COMBINATIONS OF FACTORS *f* AND *p*

Matings between black-yellows (*CCffPP* and Cc^dffPP) and animals from a pink-eyed, pale sepia-yellow stock (c^dc^dFFpp) resulted in black-eyed, black-reds (Cc^dFfPp) and black-eyed medium sepia-yellows (c^dc^dFfPp). A rather extensive 3-factor F_2 generation was bred ($Cc^dFfPp \times Cc^dFfPp$) and also a considerable number from 4 matings of type ($Cc^dFfPp \times c^dc^dFfPp$) (table 8). Two new color varieties appeared, namely, a mosaic of pale cream and yellow with pink eyes, and a pink-eyed nearly self white variety usually with small amounts of pale cream in the fur. Breeding tests demonstrated that these were the desired combinations *C–ffpp* and c^dc^dffpp. As neither showed any trace of sepia in the fur it becomes necessary to suppose that *ff* in combination with *pp* has an effect on black, contrary to the expectation based on the combinations of *ff* discussed earlier. This effect seems to be one of complete elimination, permitting an underlying pale cream (in *C–ffpp*) to reveal itself. The latter seems to be as pure a cream as that in eec^dc^dffPP, from which indeed it is virtually indistinguishable in grade of intensity.

It was not clear at first whether the pale cream or the yellow areas in the mosaics were to be considered as representing the "black" portion of the tortoise-shell pattern. The production of agoutis of composition *A–C–ffpp* settled the matter. In these, each agouti hair was pale cream or white with a yellow subterminal band indicating that the former colors represented black and the latter red. The reduction of red merely to yellow in *C–ffpp* as in *CffPP* is in harmony with the absence of effect of *p* on yellow pigmentation. In the pink-eyed pale cream and white variety found to be of constitution c^dc^dffpp, it appears that the white represents the black of the tortoise-shell pattern and the pale cream the red parts.

A stock consisting of pink-eyed cream-yellow mosaics and pink-eyed white-pale creams was produced from F_3 matings. One of the F_2 and two of the F_3 pink-eyed cream-yellows were tested by mating with stock pink-eyed pale sepia-yellows (c^dc^dFFpp). In each case intense young with red in the fur were produced proving that the tested animals carried factor *C* and were therefore of composition *C–ffpp*. An apparent pink-eyed white

from $c^dc^dFfpp \times Cc^dffpp$ was mated with a medium sepia-pale cream (c^dc^dffPP) and produced 5 young all like the medium sepia-pale cream parent in harmony with the hypothesis that it was c^dc^dffpp.

TABLE 9

The segregation in F_2 following matings of type $CCffPP \times c^rc^rFFpp$.

FUR COLOR		EYE COLOR	GENETIC CONSTITUTION	$Cc^rFfPp \times Cc^rFfPp$	
"Black" areas	"Yellow" areas			Observed	Calculated
Black	Red	Black	CFP	15	11.0
Dark Sepia	White	Red	c^rc^rFP	1*	3.7
Black	Yellow	Black	$CffP$	3	3.7
Pale Sepia	Red	Pink	$CFpp$	1	3.7
Dark Sepia	White	Red	c^rc^rffP	1*	1.2
Pale Sepia	White	Pink	c^rc^rFpp	2	1.2
Pale Cream	Yellow	Pink	$Cffpp$	2	1.2
White	White	Pink	c^rc^rffpp	1	0.4
				26	26.1

* As c^rc^rFP and c^rc^rffP are indistinguishable, the two red-eyed dark sepia-white young from this mating may have been either of these types.

A mating of type $c^rc^rFFpp \times CCffPP$ (table 9) gave the basis for three-factor F_2 matings in which c^r took the place of c^d in the matings discussed above. One of these matings produced in F_2 an apparent albino which, however, must have been of constitution c^rc^rffpp, indicating that ff combined with pp eliminates black in a variety in which yellow is eliminated by c^rc^r. Two red-eyed medium sepia-whites were produced which may have been either c^rc^rFP or c^rc^rffP. The appearance of two pink-eyed cream-yellows confirmed the view that these are of constitution $Cffpp$.

TABLE 10

Distribution of grades of cream (in place of sepia) in animals of constitutions ffppB- and of different known constitutions relative to the albino series.

GENETIC CONSTITUTION	WHITE	CREAM IN PLACE OF SEPIA $ffppB$				NUMBER	MEAN	σ
		Pale cream		Cream				
	0	1	2	3	4			
$C-$		4	4	4	1	13	2.15	0.95
c^dc^d	16					16	0.00	0.00
c^rc^r	1					1	0.00	0.00

The close similarity of the intensity of the pale cream ($e^pe^pc^dc^dffpp$) (grade 1.6) representing the yellow parts of the tortoise-shell pattern and

of that of e^pe^pC–$ffpp$ (grade 2.2) representing the *black* parts indicates the possibility of a rather peculiar two factor ratio. There can be no reasonable doubt that both eec^dc^dffpp and $EECCffpp$ would be pink-eyed pale creams of about grade 2. True breeding stocks of the two sorts should be indistinguishable. Crosses yielding pink-eyed pale creams of type $EeCc^dffpp$ would give no indication of a genetic difference. In F_2, however, one would expect a 3:10:3 ratio of yellows (eeC–), pale creams (E–C– and eec^dc^d) and whites (E–c^dc^d), all pink-eyed.

TABLE 11

Distribution of grades of yellow in animals of constitution Ff and of different known constitutions relative to the albino series.

GENETIC CONSTITUTION	WHITE	GRADE OF YELLOW *Ff*												NUMBER	MEAN	σ
		Pale Cream		Cream			Yellow			Red						
	0	1	2	3	4	5	6	7	8	9	10	11	12			
C–									2	40	220	15	3	280	9.92	0.50
c^kc^a					6	1								7	4.14	0.35
c^dc^d					2	1	9	14	2					28	6.46	0.96
c^dc^r				1	12	3								16	4.13	0.47
c^dc^a				1	13	7								21	4.29	0.54
c^rc^r	X													X	0.00	0.00
c^rc^a	X													X	0.00	0.00
c^ac^a	X													X	0.00	0.00

IMPERFECT DOMINANCE OF *f*

In the majority of cases, heterozygotes (Ff) are indistinguishable from homozygous (FF) litter mates. This applies to all albino series compounds examined. The averages (table 11), however, indicate that dominance is not quite perfect. In the case of C–Ff the average is 9.9 compared with 10.6 for C–FF and in the case of c^dc^dFf an average of 6.5 is to be compared with one of 7.0 for c^dc^dFF (table 16). The numbers are smaller in the other cases and the differences not consistent. The stocks involved are so nearly the same that the significant difference indicated by the probable errors in these cases apparently can only be due to imperfect dominance. In the first of these cases, grade 9 is very uncommon in homozygotes, but in heterozygotes 40 out of 280 were assigned this grade while 2 others were actually assigned grade 8 a "yellow" rather than a "red." On the other hand, a number of heterozygotes were assigned grades of 11 and 12, indicating that there is no necessary dilution.

There is also evidence (table 12) for imperfect dominance in the effect on black in the presence of factor *pp*. The sepia of stock pink-eyed pale sepia-yellows (*c^dc^dppFF*) is always markedly distinct from yellow of any grade of intensity. The pink-eyed pale sepia yellows which appeared in the 3-factor F_2 matings, discussed above, were not nevertheless always easy to distinguish from the pink-eyed cream-yellows. The sepia parts of many were of a creamy color differing from the cream of the latter only in a slight sootiness. The two varieties were, of course, easily distinguished

TABLE 12

Distribution of grades of pale sepia in animals of constitutions FfppBB and of different known constitutions relative to the albino series.

GENETIC CONSTITUTION	WHITE	GRADE OF PALE SEPIA (YELLOWISH) *FfppBB*												NUMBER	MEAN	σ
	0	1	2	3	4	5	6	7	8	9	10	11	12			
C–						3	0	6	6	3	2			20	7.60	1.43
c^dc^d			1	4	5									10	3.40	0.66
c^dc^r			1		1									2	3.00	1.00

by breeding tests designed to reveal the presence of factor *C* or of factor *F*. The average grade of sepia in *c^dc^dFFpp* is 4.9, in *c^dc^dFfpp* was 3.4, still on the sepia scale but so close to cream (of slightly higher grade) as to be difficult to grade. The average grade of sepia in *C–FFpp* was 9.7, with a strongly slaty quality, the average grade of *sepia* in *C-Ffpp* was 7.6 and much browner in quality, the average grade of *cream* representing sepia in *C–ffpp* was 2.2.

ABSENCE OF LINKAGE

The results shown in tables 8 and 9, combined in table 13, are so close to the expected ratios that it seems reasonably certain that there is no linkage between any two of the three factors c^d, *f* and *p*.

It may be added that fairly extensive data indicate that there is no linkage among the factors *s*, e^p, *a*, c^k, *p* and *b* and that *f* at least crosses over freely with *s*, e^p, *a* and *b* as well as with c^d and *p*.

ENVIRONMENTAL EFFECTS

In addition to the genetic factors which affect intensity and quality of color, there are environmental ones which may be considered here briefly. The sepia parts of the fur in all of the albino series compounds become darker as the animal grows older, often becoming a dark sepia or black. This effect is especially marked in red-eyed sepias. Even albinos develop

TABLE 13

Combination of data in tables 8 and 9 bringing out the approach of the observed results to those calculated, on the assumption of no linkage among factors c^d, f and p. The probability from $X^2(=2.56)$ is .95 of getting as poor a fit by chance.

	GRAND TOTAL	
	Observed	Calculated
C F P	88	91.4
cc F P	43±1	44.7
C ff P	37	30.5
C F pp	30	30.5
cc ff P	17∓1	14.9
cc F pp	15	14.9
C ff pp	8	10.2
cc ff pp	4	5.0
	242	242.1

black pigmentation in the exposed parts of the skin and often enough sootiness on the back to make it possible to distinguish a piebald or white tortoise-shell pattern. Red or yellow parts of the fur, on the contrary, do not ordinarily change in intensity to any appreciable extent unless factor *f* is present. The only case of a change which the writer has noted, in a stock in which factor *F* was present, was in a particular inbred family no. 18, in which animals genetically dilutes, $c^k c^k$, were reddish yellow (between grades 8 and 9) at birth, and later changed to an ordinary yellow (grade 7). While the yellow itself does not ordinarily change a black sootiness usually appears in yellow spots with age, especially in the cream or white spots which represent the yellow portion of a tortoise-shell pattern.

With factor *f* present, sepia darkens as described above, but yellow becomes markedly paler. Yellow of grade 7 or 8 (*C–ff*) fades to cream of grade 3 or 4, or even to sooty white. Pale creams of grade 1 to 3 ($c^{kd}c^{kd}ff$), fade to sooty white. Browns (*bb*) darken slightly with age but much less conspicuously than sepias. The pale sepias found with factor *p* on the other hand usually become distinctly paler and in some cases creamier with age.

These effects are accentuated by pulling or cutting the hair and thus exposing the skin to cold. The results of a considerable number of experiments in which a patch of hair was plucked from animals, in most cases a few days after birth, are summarized in tables 14 and 15. In the case of sepias and even blacks, the spot of new hair stood out distinctly after two or three weeks because of its darker color. The greatest effect was obtained

in 4 red-eyed medium sepias in which the new hair came out nearly 5 grades darker, practically intense black. The effect was marked, however, in all cases in which the hair was not nearly black to begin with. A spot of grade 5 was brought out in one albino and a slight tinge of sepia in another. No sepia appeared in 4 other albinos but the plucked hair may have come from a piebald area in which cold never brings out pigmentation.

TABLE 14

Summary of experiments on the change of color of sepia hair following the plucking of an area.

	FORMULA	COLOR OF NEW HAIR			AVERAGE GRADE		CHANGE IN GRADE
		Lighter	No change	Darker	Unplucked	New hair	
Black	C	..	..	2	21.0	21+	+
Dark sepia	c^kc^a	..	..	2	18.0	20.5	+2.5
Medium sepia	c^dc^d	..	..	9	15.8	18.7	+2.9
Light sepia	c^dc^a	..	..	11	13.9	16.8	+2.9
Dark sepia (red eye)	c^rc^r	..	..	2	20.5	21.0	+0.5
Medium sepia (red eye)	c^rc^a	..	..	4	16.0	20.7	+4.7
White (albino)	c^ac^a	..	4	2[1]	0.0		+
Pale sepia (pink eye)	$C\ pp$	1	2[2]	..	7.0	6.0	−0.0
" " " "	$c^kc^k\ pp$	6	..	..	8.7	4.3	−4.4
" " " "	$c^kc^d\ pp$	3	..	..	7.3	5.7	−1.6
" " " "	$c^kc^r\ pp$	1	..	1	7.0	7.0	0.0
" " " "	$c^dc^d\ pp$	6	..	..	5.2	1.3	−3.9
" " " "	$c^dc^a\ pp$	2[3]	..	..	..	..	..
" " " "	$c^rc^r\ pp$	..	..	1	..	..	+
Brown	bb	..	3	5	14.0	15.0	+1.0
Pale brown (pink eye)	$bbpp$	2	4	..	5.5	5.0	−0.5

[1] One became Grade 5, the other Grade 1.

[2] Includes an old animal already faded to Grade 2.

[3] In these, pale sepia was replaced by a nearly pure cream.

Plucked areas in browns of various sorts developed hair which was in general darker than the surrounding hair. The difference was barely perceptible, however.

In pink-eyed pale sepias, the new hair was distinctly lighter than the surrounding hair in 19 cases, showed no contrast in 2 cases and seemed slightly darker in 2 cases. In a few cases it had a distinctly creamy hue.

In experiments on yellow and agouti areas, there was a lightening of the color where any change was perceptible. The effect was much more distinct with agouti than with clear yellow. Ten red agoutis had sharply

distinct yellow agouti (or light red agouti) spots after the new hair had grown. The effect was less marked in yellow agoutis and noticed in only one of three cream agoutis. Plucking did not bring out any yellow in the white brindle spots of red-eyed sepias, but did bring out sepia sootiness in two of three animals tested.

TABLE 15

Summary of experiments on the change of color of agouti and yellow hair following the plucking of an area.

		COLOR OF NEW HAIR			AVERAGE GRADE		CHANGE IN GRADE
	Formula	Lighter	No change	Darker	Unplucked	New hair	
Agouti (red band)	C	10	..	..	11.0	7.7	−2.3
Yellow agouti	c^kc^k	5	2	..	7.0	5.6	−1.4
Cream agouti	c^kc^a	1	2	..	..	..	..
Red	C	4	2	..	11.0	10.0	−1.0
Yellow	$c^{kd}c^{kd}$	2	4	..	7.0	6.3	−0.7
Cream	$c^{kd}c^a$	..	2	..	5.0	5.0	0.0
White (tortoise)	c^rc^a	..	3[1]	..	0.0	0.0	0.0
Yellow agouti	Cff	2	..	..	..	..	..
Yellow	Cff	3[2]	1	..	..	..	..

[1] No yellow appeared, but in two cases marked sootiness developed.

[2] Whole fur changed to cream with spot leaving no contrast.

In yellow agoutis and yellows in which the dilution of color is due to *ff*, the whole fur fades in about two months. Plucking, however, usually brings out a contrasting spot.

In all of these cases the contrasting spot disappears after several months when all of the fur has been replaced.

The experiments described above were carried on throughout most of a year (January to October). Marked effects were obtained in summer months as well as in winter months, indicating that no great fall in skin temperature is necessary.

That the pigmentation which develops in the naturally exposed parts of the skin of albino guineapigs and in parts from which the hair has been removed are due to cold rather than to some other stimulus is probable from the experiments of SCHULTZ (1922) and ILJIN (1926a) with albino guineapigs as well as from the experiments of SCHULTZ (1918), LENZ (1923), KAUFMAN (1925) and ILJIN (1926b) in the similar case of the Himalayan rabbit. It is also probable that the development of black sootiness in tortoise-shell spots in guineapigs is comparable to the blackening of the sooty yellow (or Thuringian) rabbit which SCHULTZ (1922) has also shown to be due to cold.

The variation in grade of color at birth among animals of the same factorial composition, as far as known, seems to be largely non-genetic in origin. A portion must be attributed to variations in judgment of colors, but this can only rarely account for differences of more than one grade among individuals. The standard deviations are given in the last columns of the tables which present the distributions, and are averaged by groups in table 16.

It will be noticed that the effects of the environmental factor cold (to which the ordinary age effect is probably due) are more complex than

TABLE 16

Averages of standard deviations of classes whose distributions are given in tables 3 to 7 and 10 to 12 of this paper and tables 11 and 12 of the preceding (Wright, 1925). The groupings by mean grade and constitution are those which seem of most significance. These averages have been used in calculating probable errors.

CLASSES (BY MEAN GRADE AND CONSTITUTION)			NUMBER CLASSES	NUMBER ANIMALS	AVERAGE
Black	(21)	(*C-ffP*)	1	151	0.32
Dark sepia	(18–20)	(*P*)	7	605	0.80
Medium sepia	(11–18)	(*P*)	5	634	1.26
Pale sepia	(4–11)	(*pp*)	8	345	1.07
Very pale sepia	(0–4)	(*pp*)	4	84	0.75
Brown	(11–18)	(*P*)	10	539	1.03
Pale brown	(4–11)	(*pp*)	6	345	0.83
Very pale brown	(0–4)	(*pp*)	4	54	0.70
Red	(9–13)		2	519	0.57
Yellow	(6–8)	*FF*	3	176	0.50
"	"	*C-ff*, c^dc^d *Ff*	2	290	0.78
Cream	(3–6)	c^kc^a *FF*	2	251	0.71
"	"	c^dc^a *FF*	2	383	0.44
"	"	$c^{kd}c^a$ *Ff*	3	44	0.48
Pale cream	(1–3)	*ff*	3	107	0.87
Creamy white	(0–1)	*ff*	3	104	0.38

those of any genetic factor, even *f*, which we have considered. It affects the sepias with *P* present and those with *p* in opposite directions. It accentuates the dilution effect of *f* on yellow. It dilutes yellow markedly in agouti hairs but only slightly if at all in non-agouti hairs. The indication is that temperature affects in one way or another many independent processes which have to do with pigment production, while the genetic factors are more specific, each affecting only one or at most only a very few processes.

DISCUSSION

In attempting to analyze the physiological workings of genetic factors, we must distinguish between primary and secondary effects. It must be supposed that there are one or more conditions in the cells of a developing organism under which each gene has some immediate physiological action. The smallness of genes and their persistence has suggested to many (MOORE 1910, GOLDSCHMIDT 1916, TROLLAND 1917, and others) that in this action the genes must behave as enzymes. The primary products themselves become factors in later developmental processes and so on in ever widening circles. Through the mediation of the circulatory system, nervous system, as well as through mechanical influences, a single primary gene action may ultimately have effects on the most diverse characters. It is to be expected that the relation between factor and character will ordinarily be found to involve a great many steps. There is some reason to believe, however, that the relation is relatively close in the case of melanin pigmentation.

The melanin pigments are amorphous, organic compounds containing nitrogen, the exact chemical constitutions of which are as yet unknown. The generally accepted theory as to their origin traces to the work of BERTRAND in 1896, who was able to extract an oxidizing enzyme from plant tissues which converted tyrosin into a substance resembling the natural melanins in its properties. Tyrosinase has since been demonstrated in the tissues of many invertebrates by a considerable number of investigators. As to vertebrates, DURHAM (1904) obtained indication of tyrosinase in the skin of colored guinea pigs. H. ONSLOW (1915) was unable to confirm her results by the same method but obtained a dark melanin-like substance by adding tyrosin and hydrogen peroxide to a colorless extract from the skin of young black rabbits. He was unable to extract such a peroxidase from white skin, whether from albinos or white spotted animals, or from the skins of yellow rabbits. The extracts in these three cases did not prevent the reaction when added to extracts from black rabbits. The reaction was, however, inhibited by extracts from the skin of dominant whites (English rabbits) or from the white belly of rabbits with the dominant agouti pattern. KAUFMANN (1925) has confirmed ONSLOW to the extent of never being able to extract tyrosinase from albino rabbits while usually obtaining positive, although slight reactions, from extracts from black rabbits.

SCHULTZ (1925) has also obtained evidence of enzyme differences in skins of rabbits of various colors. He obtained a marked blackening of "dopa" (dioxyphenylalanin), a substance more easily blackened than

tyrosin (p-monoxyphenylalanin) in frozen sections of the skin of black rabbits, a weaker *blackening* in the case of brown rabbits, a still weaker blackening in the case of yellow rabbits, whether recessive or dominant (agouti), and no reaction in the case of either recessive white (albinos, white Viennas, white parts of Dutch rabbits, or Himalayans) or dominant whites (English). The skin of Himalayans stimulated to the production of black pigment by cold, on the other hand, gave strong blackening with dopa and the same was true of sooty yellow stimulated to black production by cold. Because of the *blackening* of brown skin, he believes that dopa cannot be the actual chromogen in these cases but merely that its blackening indicates the degree of activity of enzymes which in nature oxidize a closely related chromogen.

While tyrosin has long been considered the most probable chromogen in the vertebrate skin, the question is still open. Its presence has been demonstrated in invertebrates (v. FÜRTH in PRZIBRAM, DEMBOWSKI and BRECHER 1921). An attempt to demonstrate it in vertebrate skin by SATO and BRECHER (1925) gave negative but inconclusive results. Dopa was suggested as a probable chromogen by BLOCH in 1917. PRZIBRAM (1924 and earlier) has succeeded in demonstrating its presence in the cocoons of certain insects but extensive experiments with fish scales, feathers, hair and skin of many vertebrates by SATO and BRECHER (1925) and BRECHER and WINKLER (1925) seem definitely to rule it out as the vertebrate chromogen. Other substances, more or less related to tyrosin such as adrenalin and tryptophan have been suggested as possible natural chromogens.

There is no agreement as yet as to the details of the enzyme-chromogen reaction even in those cases in which the chromogen is known. MURIEL WHELDALE ONSLOW (1923) finds reason for the suggestion that tyrosinase from plants is a mixture of enzymes including a reductase, a carboxylase, an oxygenase and a peroxidase. Suggestions have been made that the extent to which the reaction proceeds in different cases may be controlled by differences in p_H (inhibition by acid, PRZIBRAM, DEMBOWSKI and BRECHER 1921) which may in turn be affected by temperature (KAUFMANN 1925) and also that temperature may affect pigment production through the thermolability of certain of the enzyme components (LENZ 1923).

GORTNER (1911, 1912) distinguished between a diffuse pigment characteristic of red or reddish brown hair, soluble in dilute acid and containing no ash, and granular pigment from black hair (rabbit, etc.) insoluble in dilute acid and leaving 2 or 3 percent ash, chiefly iron oxide. The

relation between the enzyme differences of ONSLOW and SCHULTZ and this presence or absence of iron is an interesting question.

Most of the earlier authors believed that melanin pigment was formed in the nucleus of cells and extruded into the cytoplasm. According to HOOKER (1915) pigment is formed in the cytoplasm, especially in the immediate vicinity of the nucleus and presumably under the influence of oxidases from the latter. That the surface of contact between nucleus and cytoplasm is the chief region of intracellular oxidation had previously been demonstrated for a variety of types of living cells by R. S. LILLIE (1902). RÉNYI (1924) strongly confirms the view that pigment is formed in special bodies in the cytoplasm and finds that these bodies (Pigmentbildner) have all the staining properties of mitochondria and can be traced step by step from undoubted mitochondria. It is interesting to note that he finds Pigmentbildner in white as well as colored skin. This is in harmony with the view that the essential difference in this case is in the nuclear rather than the cytoplasmic component.

Experiments on the transplantation of skin have in general indicated autonomy in the pigment producing cells (CARNOT and DEFLANDRE 1896, LEO LOEB 1897). That there may also be diffusible components is, however, suggested by KOPPÁNYI'S (1923) experiments in which he found a development of pigmentation in the eyes of albino axolotls transplanted to pigmented hosts. Cell autonomy with regard to the effects of factors *A*, *C*, *F* and *P* in the guinea pig is indicated by the persistence of mutant spots (WRIGHT and EATON 1926). There is, of course, no necessary contradiction between cell autonomy with respect to the effects of some factors and diffusion of the effects of other factors.

On the whole, the various lines of evidence seem to be converging toward a fairly simple interpretation of the mode of action of the major genetic color factors. The most plausible hypothesis as to the genes themselves seems to be that they are to be looked upon as chemical units not much if at all beyond the size of protein molecules (MORGAN 1922, 1926), arranged in a definite linear order in the chromosomes, and characterized especially by the power of somehow duplicating themselves from the building stones in their medium, following mitosis; of simultaneously separating from these duplicates under the cell condition of mitosis; and of simultaneously attracting their homologues under the conditions of the maturation prophase. As factors in development, the genes apparently behave as catalysts in not being used up themselves by the reaction for which they are responsible. In the case of color production, the effects seem to take place through the mediation of enzymes produced by the

genes in the nucleus but acting upon protein decomposition products related to tyrosin in special bodies (mitochondria) in the cytoplasm of the appropriate cells. These enzymes, actually extractable from the skin of pigmented animals, are not, of course, to be identified with the genes themselves. Their production may or may not be related to the process by which the genes duplicate themselves between cell divisions.

Much must be learned before any such hypothesis ceases to be of a highly speculative nature. In any case, it appears probable that the relation between the major color factors of mammals and the visible color differences is exceptionally simple and worthy of thorough study from the physiological and chemical sides. The effects of the factors in the various combinations reveal a body of facts which must be taken into account in any attempt to trace the steps.[1]

RELATIONS OF FACTOR EFFECTS

On the principle of Occam's razor it is desirable to attempt analysis of factor effects by attributing as few primary effects to genes as possible and preferably only a single one. The following suggestions are essentially an analysis of the consequences of the assignment of a single graded series of primary effects as back of the apparently nonlinear effects of the albino series of factors. It will be seen that on this basis each of the factors dealt with here need have only a single point of action on color production with the exception of the gene *F* and the factors sex and temperature.

The first step in analyzing the character melanin pigmentation is the recognition of the two main series of colors, the yellow series and the sepia or black series probably corresponding to GORTNER'S two types of melanin. The processes of production clearly have some degree of physiological independence, but not complete independence. Factors with a single primary effect may act (1) on yellow production alone or (2) on sepia production alone (possible examples *B*, *P*) or (3) may determine between yellow and sepia production (possible examples *E*, *A*) or (4) may act upon both sepia and yellow production through a single process antecedent to both (possible examples *C*, *S*).

The peculiar effects of the albino series (C, c^k, c^d, c^r, c^a) in the presence of factors *FPB* have been discussed previously. In a general way the series determines differences in quantity, irrespective of quality of pigment, and thus appears to fall in the last of the above classes. The effects of the 5 allelomorphs are not the same, however, for yellow and sepia, or

[1] Since the above was written the writer has had the opportunity of reading *Goldschmidt's* highly illuminating "Physiologische Theorie der Vererbung" (1927). As may readily be seen the present discussion is in harmony with his general viewpoint.

even for sepia in eye and hair. The differences in order of effect on yellow and sepia is brought out here in figures 2 and 6. The average grades of color in these and other combinations are summarized in tables 17, 18 and 19. Factor *C* is a condition for the most intense colors of both series (red and black), factors c^k and c^d determine very nearly the same grade of dilute yellow but widely different intensities of sepia. With factors c^r and c^a no yellow whatever develops but with c^r there is an even more intense sepia than with c^d in the fur (less intense in the eyes), while with

TABLE 17

Average grades of sepia and brown in the indicated combinations of factors from tables 3, 4, 5, and 7, of this paper and table 11 of preceding. Probable errors (.6745 σ/n) based on average standard deviations of table 16.

	FPB SEPIA	*FPbb* BROWN	*FppB* PALE SEPIA	*Fppbb* PALE BROWN	*ffPB* SEPIA	*ffPbb* BROWN
C—	21.00	15.63± .07	9.70± .11	8.29± .21	20.91± .02	16.00± .68*
c^kc^k	20.10± .08	14.61± .07	9.08± .08	7.22± .04	19.00± .54*	
c^kc^d	19.43± .10	15.37± .13	7.64± .22	6.00± .18	..	
c^rc^r	20.46± .06	14.76± .10	7.00± .23	6.12± .07	..	
c^kc^a	18.53± .03	13.60± .08	5.47± .19	5.57± .11	18.00± .38	
c^dc^d	16.88± .09	14.18± .08	4.88± .07	5.43± .21	16.79± .12	15.00± .68*
c^dc^r	19.09± .05	15.00± .11	4.00± .10	3.67± .27	18.56± .18	
c^dc^a	14.02± .05	12.46± .14	2.57± .09	3.17± .19	13.91± .10	
c^rc^r	20.09± .07	15.47± .18	2.58± .10	2.82± .12	..	
c^rc^a	15.49± .07	13.06± .10	0.73± .13	1.11± .09	..	
c^ac^a	0.00	0.00	0.00	0.00	0.00	0.00

* Grade of one individual. Probable error is quartile distance (.6745σ) for groups of similar average grade.

c^a there is no visible sepia at birth though a small amount develops later in the skin and fur under the influence of cold. It was shown that these irregularities in order could be harmonized with the hypothesis that the albino series determines a graded series of effects in the order c^a, c^r, c^d, c^k, *C* on a *single* process essential to all pigmentation by means of two subsidiary hypotheses.

(1) That there is a different *threshold* of effectiveness of the immediate product of the genes of this series depending on whether the process is to go in the direction of sepia or yellow (as determined by other factors). It was shown that this threshold effect must be considered as applying to the immediate products of the two genes in the zygote *separately* instead of jointly. The difference between the order of intensity of sepia in fur and eyes requires the assumption of different regional thresholds.

TABLE 18

Average grades of yellow in the indicated combination of factors. From tables 6 and 11 of this paper and 12 of preceding. Probable errors based on average standard deviation of table 16.

	FF YELLOW	*Ff* YELLOW	*ff* YELLOW
C—	10.57± .03	9.92± .02	6.77± .03
$c^k c^k$	7.10± .05	..	1.20± .26
$c^k c^d$	7.17± .06	..	..
$c^k c^r$	4.63± .06	..	..
$c^k c^a$	4.59± .03	4.14± .12	0.00
$c^d c^d$	6.97± .04	6.46± .10	1.60± .06
$c^d c^r$	4.08± .03	4.13± .08	0.22± .09
$c^d c^a$	4.16± .02	4.29± .07	0.11± .03
$c^r c^r$	0.00	0.00	0.00
$c^r c^a$	0.00	0.00	0.00
$c^a c^a$	0.00	0.00	0.00

TABLE 19

Average grades of pale sepia or of yellow in the indicated combinations of factors. From tables 4, 12, and 10. Probable error based on average standard deviation of table 16.

	FFppBB PALE SEPIA	*FfppBB* PALE YELLOW SEPIA	*ffppBB* CREAM
C—	9.70±0.11	7.60±0.16	2.15±0.16
$c^d c^d$	4.88±0.07	3.40±0.16	0.00
$c^d c^r$	4.00±0.10	3.00±0.36	..
$c^r c^r$	2.58±0.10	0.00	0.00

(2) That above this threshold there is competition between the yellow and black processes within those portions of the fur in which the black process goes on.

Superficially, the brown series (*B*, *b*) and the pink-eye series (*P*, *p*) seem to have closely similar effects. Neither has any visible effects whatever in yellow parts of the fur but each modifies sepia wherever it occurs in fur, skin or eyes. The combination with albino series compounds show, nevertheless, that their actions must be fundamentally different. The order of intensity in the fur of these compounds is not altered by replacing. *B* by *b*, with minor exceptions which cannot be relied upon (figure 3). The same statement applies to the different order found in the eyes. The eyes of browns have brown irides in *C*, c^k, and c^d, have conspicuously less pigment in $c^r c^r$ and especially $c^r c^a$, and are without pigment (pink) in $c^a c^a$. It must be concluded that *b* modifies pigmentation of the sepia series *in such a way as not to affect the thresholds of sepia and yellow or the competition between them.*

The replacement of P by p, on the other hand, alters the order of intensity of sepia of the fur among the different albino series compounds (figure 4). It may be seen that c^r compounds are decidedly less intense than the c^d compounds in contrast with the reverse order in the presence of P. The marked difference between the c^k and the c^d compounds and the fact that c^r produces more pigment than c^a distinguishes the order from that found in the yellow part of the fur. The different apparent orders of effectiveness of the albino series genes in different genetic backgrounds are illustrated in figure 8 by comparison of the five homozygotes of the albino series. The order found with p agreeing neither with that of sepia in the presence of P or with yellow, and only doubtfully with that of eye color in the presence of P, strengthens the view that the apparently nonlinear effects of the albino series are to be attributed to secondary physiological processes, such as the suggested threshold and competition effects instead of to primary effects of the genes themselves. Specifically, the change in position of the c^r compounds suggests that p raises the threshold of sepia from a point close to the gene level of c^a (below or above depending on temperature) nearly to c^r, besides greatly weakening the pigment producing powers of the substance involved.

It might be expected that under these conditions the yellow producing process would tend to overwhelm the reduced sepia. Actually, however, there is little difference in quality between the sepias of formula $CFpB$ and those of formula c^dc^aFPB or even c^rc^aFPB which happen to be of the same grade (sepia 11 or 12) although red develops to full intensity in the appropriate regions of the first, only to the cream level in the second, and not all in the third. Indeed the pale slaty appearance of most of the p-sepias might suggest that there is no competition with yellow at all. The existence of a competition is best shown by the difference in quality of c^dp combinations in comparison with either c^kp or c^rp combinations. The former are, as previously noted, decidedly yellower in appearance. The actual combination of colors necessary to match a number of typical animals of these and other combinations on the color top are given in table 20. It will be seen that the c^dp combinations require an average of 17 percent yellow where the c^kp and c^rp compounds require only about 11 and 13 percent, respectively. The amount of orange is about 7 percent or 8 percent in all of them. The c^dp combinations are thus intermediate between the other p-sepias and the creams. These qualitative differences are presented graphically in figure 9. They may be explained on the same basis as the lower intensity of sepia in c^dP in comparison with c^rP and c^kP, that is, that with c^d present, there is competition with yellow which

is wholly lacking with c^r and not appreciably greater in c^k although the power of producing sepia is distinctly stronger in the latter. It must be concluded that in spite of the great reduction in pigment producing power when p is present, competition with yellow is as effective above the sepia

TABLE 20

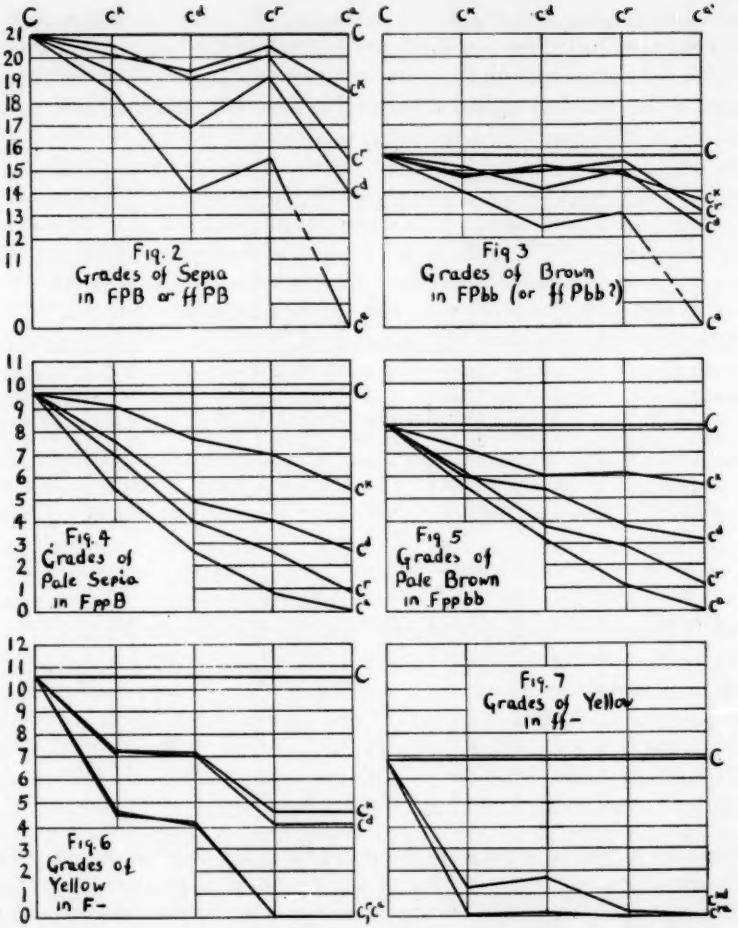
The proportion of white (W), yellow (Y), orange (O) and black (B) on the Milton Bradley color wheel which matched the sepia or brown of animals of the genetic constitution indicated.

GENETIC CONSTITUTION	NUMBER	W	Y	O	B	Y+O	$\frac{O}{Y+O}$
C-FFppB-	3	11.3	10.0	7.7	71.0	17.7	43.5
C- "	5	7.2	6.4	5.0	81.4	11.4	43.9
c^kc^k "	5	16.6	9.8	6.6	67.0	16.4	40.2
c^kc^d "	2	21.5	10.5	8.5	59.5	19.0	44.7
c^kc^r "	3	15.0	10.7	8.0	66.3	18.7	42.8
c^kc^a "	6	25.7	12.3	8.0	54.0	20.3	39.4
c^dc^d "	7	33.3	17.9	8.1	40.7	26.0	31.2
c^dc^r "	5	31.4	15.8	6.4	46.4	22.2	28.8
c^dc^a "	1	30.0	18.0	11.0	41.0	29.0	37.9
c^rc^r "	3	37.0	12.0	7.3	43.7	19.3	37.8
C-FfppB-	2	17.5	13.0	8.5	61.0	21.5	39.5
C-FFppbb	4	13.2	11.8	9.7	65.3	21.5	45.1
c^kc^k "	4	16.8	12.0	9.7	61.5	21.7	44.7
c^kc^d "	4	15.0	15.5	11.0	58.5	26.5	41.5
c^kc^r "	3	18.3	13.0	10.3	58.3	23.3	44.2
c^rc^r "	1	28.0	12.0	10.0	50.0	22.0	45.5

threshold as when P is present. Summing up, p has an effect on the sepia process which involves a raising of the threshold, a great reduction in pigment producing power but no reduction in the power of competing with yellow except that due to change of threshold.

It may be noted that combination of b with p (figure 5) does not change the order of sepia among the albino series compounds as found with Bp (figure 4) but in the main merely changes the quality. This confirms the view that b has a modifying influence which is independent of the threshold and competition effects.

The most conspicuous effect of factor f is the reduction of intensity of yellow. The sequence of albino series compounds is unaltered as far as



FIGURES 2–7.—The average grades of fur color found with each combination of factors of the series *F*, *P* and *B* with different combinations in the albino series. Each line in each graph represents the grades found when the albino series gene at the right end of the line in question is combined successively with the albino series genes at the top of the figures.

FIGURE 2.—Grades of sepia in *F P B*. There is no significant difference in *ffP B*.

FIGURE 3.—Grades of brown in *F P bb*. Probably no difference in *ffPbb*.

FIGURE 4.—Grades of pale sepia in *F ppB*.

FIGURE 5.—Grades of pale brown in *F ppbb*.

FIGURE 6.—Grades of yellow in *FF*.

FIGURE 7.—Grades of yellow in *ff*. Combinations $c^k c^d$, $c^k c^r$ not produced.

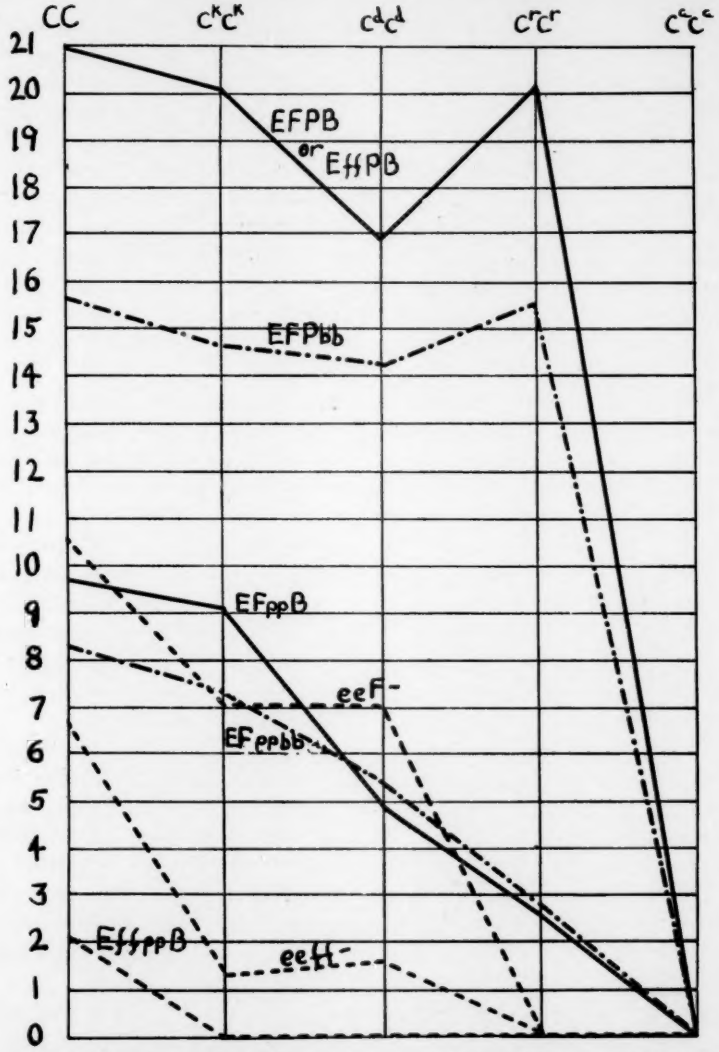


FIGURE 8.—The average grades of sepia (solid), brown (dot and dash), or yellow (dash) found with particular combinations of factors of the series *F*, *P* and *B* with the 5 homozygotes of the albino series. Factor *E* is used to indicate reference to "black" parts of the fur and *e* to "yellow" parts of the fur, although for the most part the actual grades were obtained in both cases from tortoise-shells (e^p).

these have been produced, as may be seen by comparison of figure 6 and 7. The reduction can not, however, be attributed to a lowering of the threshold of the *immediate* gene products, since c^k and c^d in compounds with c^r and c^a ordinarily produce no yellow pigment at all while when homozygous they produce a pale cream. There is no apparent effect on *PB* sepias (table 17) or *Pb* browns. Inasmuch as competition with yellow has been assigned as one of the factors in explaining the peculiar order in these cases, the absence of change, accompanying marked reduction of yellow by *f*, indicates that the latter process may follow and in any case must be independent of the competition effects. The most striking instance is that of c^dc^aFPB with sepia of grade 14.0, cream of grade 4.2 which remains sepia of grade 13.9 on replacing *F*– by *ff*, although the cream is typically completely eliminated.

While factor *f* seems to have neither primary nor secondary effects on the intensity of *P*-sepias, it completely eliminates *p*-sepias revealing an underlying cream. The fact that the cream, in those spots of the tortoise-shell which represent black, is paler than the yellow in those of the spots which represent red (and similarly in the black and red bands of the agouti patterns) indicates two things: first, that the competition effect is present in spite of the complete absence of visible sepia and, second, that the competition effect involves a reduction of yellow as well as the reduction of black which it was previously advanced to explain. As with the effect on yellow pigmentation, it must be concluded that the effect of factor *f* on *p*-sepia either follows or at least occurs independently of the competition effect.

The fact that *f* leaves pale cream (grade 2.2) in place of black in animals in which red is reduced merely to yellow of grade 6.8 gives some indication of the normal extent to which the black producing process interferes with yellow in the "black" region. Factor *f* reduces yellow of grade 7.0 (c^dc^dF) to a pale cream of nearly the same grade (1.6) as that referred to above. It may be inferred that black production, in the absence of *f*, tends to reduce red (grade 10.6) to yellow of about grade 7 and that the pale cream actually found in "black" areas when *f* is present represents this effect with the effect of *f* on yellow added to it.

Summing up, *f* reduces yellow pigmentation and causes complete absence of the kind of sepia determined by *p*. Both effects must either follow or at least occur independently of the threshold and competition effects which determine the order of intensity of the albino series compounds.

The deductions as to processes involved in color production and the points at which the known color factors have their primary effects are

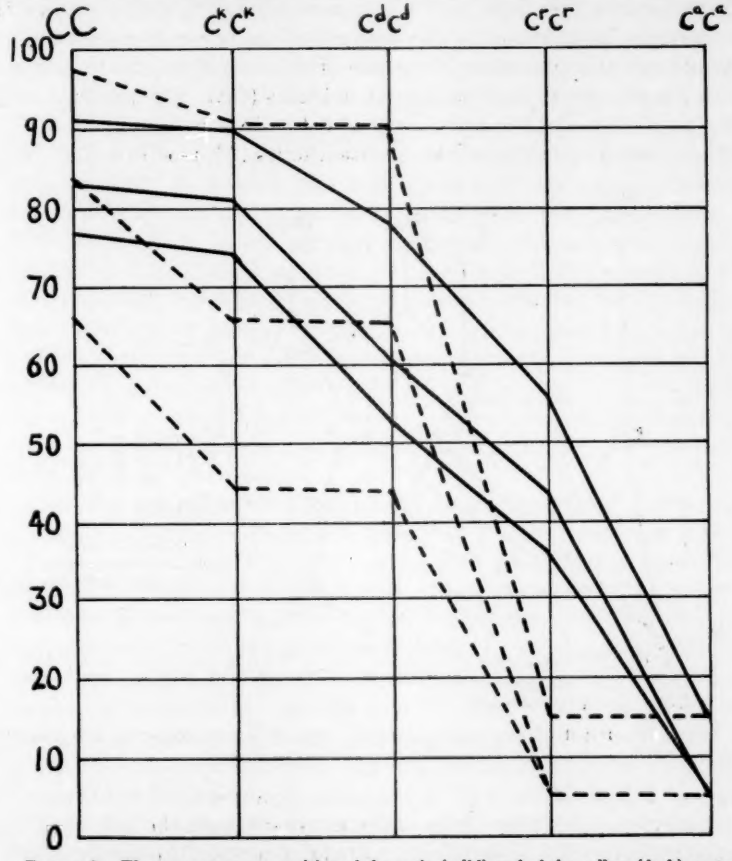


FIGURE 9.—The percentage composition of the sepia (solid) and of the yellow (dash) parts of the fur in terms of black, orange, yellow and white (reading from the bottom of the graph) in the 5 homozygotes of the albino series combined with *FFpp*B–. Note the spread in the amount of yellow in the sepia of c^dc^d in comparison with that in c^rc^r, c^kc^k, and *CC*.

presented graphically in figure 10. The albino series is represented as determining the quantity of a substance, I, called enzyme I in earlier publications (WRIGHT 1916, 1917) on the strength of ONSLOW'S determination of the presence of a tyrosinase in the skin of the black rabbit (*C*), absent in the skin of albinos (c^a). Since the "I" of the diagram is

only a precursor of ONSLOW's tyrosinase rather than the enzyme itself, it is perhaps preferable to use the more noncommittal term substance I. This substance may unite with a substance labeled II, called enzyme II in previous publications on the strength of ONSLOW's determination of the absence of a tyrosinase in the skin of yellow rabbits (*Ce*) in contrast with the presence of such an enzyme in blacks (*CE*). The presence of II in a particular region is represented as determined by a number of conditions, including factors of the extension series (*E*), modifiers (ΣE), sex,

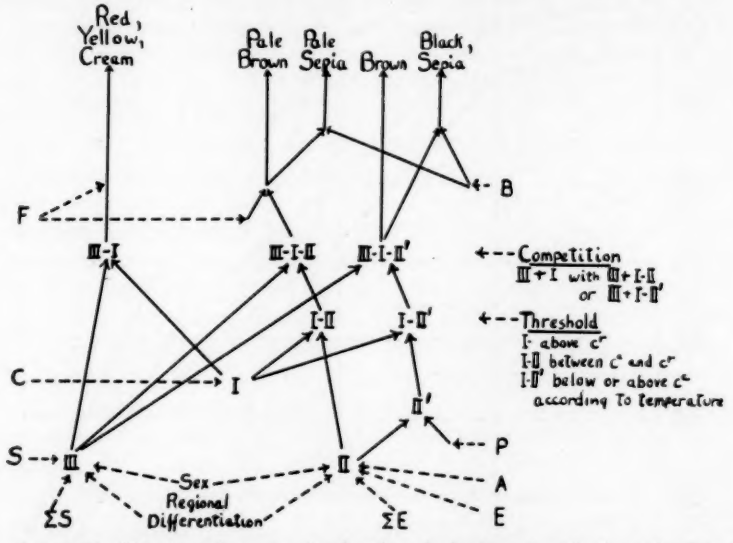


FIGURE 10.—Hypothetical processes involved in melanin pigment production and points of action of factors as deduced from the effects of factor combinations.

regional differentiation, and factors of the agouti series (*A*). ONSLOW's discovery of an inhibitor of the action of tyrosinase in the skin of agouti rabbits suggests the mode of action of the *A*-series. The nature of substance II is represented as affected by the *P*-series. The action of the *P*-series must be located at this point on the strength of the evidence that it precedes the threshold and competition effects.

The union of substances I and II is represented as essential to the production of sepia or brown pigmentation, while substance I without II is represented as essential for production of yellow. The evidence indicates that I and its products with II are ineffective below certain levels of production (of I), which vary with presence and nature of II as described.

Whatever the cause of these threshold differences, the evidence indicates that they apply to the immediate products of the two *C*-series genes in the zygotes before these products have come together in the cell (figures 2, 4 and 6, and discussion in previous paper, WRIGHT 1925). The union of I with II must of course be included among the processes which occur in the immediate vicinity of each of the *C*-bearing chromosomes.

The two substances I and I–II are represented as competing (in the regions in which II is present at all) for union with a third substance (III). It is necessary to suppose that I–II is about equally effective whether modified by *P* or *p*, except for the threshold difference. The assignment of the action of the piebald series (S_s) and accessory modifiers (genes ΣS, sex, age of dam, etc.) to substance III is arbitrary. It is merely known that something essential to all pigmentation is affected in an all-or-none fashion in different parts of the skin. This may be back of I instead of III.

The brown series (*B*, *b*) appears to act upon the precursors of sepia exclusively but regardless of modifications by *P* or *p* and without influence on threshold or competition with yellow. This effect is most conveniently represented in the diagram as following these processes, although this is not a necessary conclusion.

Two distinct effects must apparently be assigned the *F* series. The threshold of yellow production is raised by *f*, but in this case without influence upon competition. The effect must be related to the zygotic combination instead of to the *C*-series genes separately. The action is thus best represented as following the competition effect. Factor *F* is also an essential for any production of sepias in the absence of factor *P*. As the action of *p* does not prevent the competition effect, action of *f* is again most conveniently represented as following. While the *f*-series like sex has been represented as affecting color production at two points, the possibility that these both rest on a single primary action of the gene is by no means ruled out.

The differences between the proportions of yellow and orange required to match intense reds, yellows and creams (table 2) suggest that more is involved than mere differences in quantity of pigment within the yellow series of colors. The close similarity in the effects of c^k and c^d on yellow in contrast with the differences in effects on sepia, suggest the existence of a threshold between yellow of grade 7 or 8 and red of grade 10 reached at the level of c^d but not passed at that of c^k (or by *C* in combination with *f*). It has not seemed desirable to introduce these complications into the diagram. Neither has it seemed desirable to indicate the apparently multiple effects of temperature.

The processes of melanin pigment production are probably essentially the same in whatever form of life they occur. The similarity of the compounds of the albino series in the mammals most studied has been discussed in previous papers (WRIGHT 1925). The evidence for a threshold difference between sepia and yellow in the rabbit, mouse, rat, dog and cat, comparable to the situation in the guinea pig, is especially noteworthy. Most of the other known color factors of mammals have effects which are similar as far as studied to the factors of the guinea pig and thus readily fall into the scheme presented here which does not differ in its essentials from that previously suggested (WRIGHT 1916–17). The most important types of color variation not found in the guinea pig are the dominant whites, such as the factor for the English pattern of rabbits, in which ONSLOW found an inhibitor of the action of tyrosinase; and the type of dilution of all color found in the blue and creams of rabbits, mice and maltese cats. These factors, however, introduce no serious difficulties.

SUMMARY

The distribution of sepia and yellow pigments in the fur of guinea pigs is determined by factors of the series *S*, *E* and *A* and modifying factors. The quality and intensity of these pigments is largely determined by factors of the series *C*, *F*, *P* and *B*. All combinations of the *C*, *P* and *B* series have been made with *F* present, and enough have been made with *f* present to determine the main differential effects of this pair. The sepia and yellow parts of the fur in these combinations have been graded by means of standard skins. The percentage compositions of the colors of these skins are given in terms of white, yellow, orange and black on the Milton Bradley color wheel.

The albino series compounds do not fall in the same order of intensity in different combinations with other factors. In combination *FPB* and *FPb*, the albino series genes determine increasing intensity of sepia in the fur in the order c^a, c^d, c^r, c^k, *C*. In the eyes of the same animals the order is c^a, c^r, (c^d, c^k, *C*), the latter three being indistinguishable by mere inspection. In yellow parts of the fur the order is (c^a, c^r), (c^d, c^k), *C*, the first two producing no yellow whatever and c^d and c^k being almost indistinguishable. Factor *b* has no effect on yellow. It replaces black by brown, but as noted above does not change the albino series order. Factor *p* also has no effect on yellow. It greatly reduces the intensity of sepia in the fur and almost eliminates all pigmentation in the eyes. The most interesting effect is another change in the order of the albino series genes in their effect on sepia c^a, c^r, c^d, c^k, *C* in both *FpB* and *Fpb*. Factor *f* reduces the

level of yellows in all combinations not already white with factor *F*. In contrast with the preceding cases, the effect appears to be on the products of zygotic compounds rather than on those of the separate *C*-series genes. The order of the zygotes is not changed. Factor *f* has no effect on grade of sepia in combination with *P*, but completely eliminates sepia in the presence of *p*, leaving a pale cream or white in its place. Dominance of *F* is not quite perfect. There is no genetic linkage between factors *C*, *F* and *P*.

Deductions are made as to the succession of processes involved in pigmentation of the various sorts and as to the points in this scheme at which the color factors have their primary effects.

These deductions center about two hypotheses: first, that the immediate product (I) of action of the genes of the *C*-series has different thresholds of effectiveness depending on whether an accessory substance (II) necessary for any sort of sepia or brown is present or not and whether or not II if present is modified by the *P*-series; second, that above these thresholds there is competition between the precursor of yellow (I alone) and the precursor of sepia and brown (I–II). Factors *B* and *F* must be assigned effects following or otherwise without effect upon the above threshold and competition effects.

LITERATURE CITED

BLOCH, B., 1917 Chemische Untersuchungen über das spezifische pigmentbildende Ferment der Haut, die Dopaoxydase. Zeitschr. f. Physiol. Chemie. **98**: 226.

BRECHER, L., and WINKLER, F., 1925 Übereinstimmung positiver und negativer Dopareaktionen an Gefrierschnitten mit jenen an Extrakten. Arch. f. Mikr. Anat. u. Entwicklungsmech. Organ. **104**: 659–663.

CARNOT, P., and DEFLANDRE, CL., 1896 Persistence de la pigmentation dans les greffes epidermiques. C. R. Soc. de Biol. Paris, **3**: 178–180.

CASTLE, W. E., 1905 Heredity of coat characters in guinea pigs and rabbits. Carnegie Inst. Washington. Publ. No. 23. 78 p.

1907 Color varieties of the rabbit and of other rodents: their origin and inheritance. Science N. S. **26**: 287-291

1914 Some new varieties of rats and guinea pigs and their relation to problems of color inheritance. Amer. Nat. **48**: 65–73.

1916 An expedition to the home of the guinea pig and some breeding experiments with material there obtained. Carnegie Inst. Washington. Publ. No. 241. Part I: 3–55.

1925 Heredity in rabbits and guinea pigs. Bibliographia Genetica **1**: 419–458.

DAVENPORT, C. B., 1904 Statistical Methods. 225 pp. New York: John Wiley and Sons.

DETLEFSEN, J. A., 1914 Genetic studies on a cavy species cross. Carnegie Inst. Washington. Publ. No. 205. 134 pp.

DURHAM, F. M., 1904 On the presence of tyrosinases in the skin of some pigmented vertebrates. Proc. Roy. Soc. London **74**: 310.

GOLDSCHMIDT, R., 1916 Genetic factors and enzyme reactions. Science N. S. **43**: 98–100.

GORTNER, R. A., 1911 On melanin. Biochem. Bull. **1**: 207–215.

1912 On two different types of melanin. Proc. Soc. Exp. Biol. and Med. **9**: 3–4.

HOOKER, D., 1915 Roles of nucleus and cytoplasm in melanin elaboration. Anat. Rec. **9**: 393–402.

IBSEN, H. L., 1916 Tricolor inheritance. I. The tricolor series in guinea pigs. Genetics **1**: 287–309.

ILJIN, N. A., 1926 Studies in morphogenetics of animal pigmentation. I. Morphogenetic analysis of the genetical constitution in albino guinea pigs. Trans. Lab. Exp. Biol. Zoopark of Moscow. **I**: 1–10.

II. Investigation of the temperature influence of the Himalayan rabbits pigmentation. Trans. Lab. Exp. Biol. Zoopark, Moscow. **1**: 11–63.

KAUFMANN, LAURA, 1925 An experimental study on the partial albinism in Himalayan rabbits. Biologia Generalis **1**: 7–21.

KOPPÁNYI, THEODOR, 1923 Die Replantation von Augen. VI. Wechsel der Augen und Körperfarbe. Arch. f. Entwicklungsmech. Organ. **99**: 76–81.

KOSSWIG, CURT, 1925 Die Vererbung von Farbe und Zeichnung bei Nagetieren. Zeitschr. f. Tierzüchtung **5**: 101–129.

LENZ, F., 1923 Die Akromelanie der Russenkaninchen und ihre Bedeutung für unsere Auffassung von der Akromegalie. Arch. Rass.–Gesellschaftsbiol. **15**: 24–33.

LILLIE, R. S., 1902 On the oxidative properties of the cell nucleus. Amer. Jour. Phys. **7**: 412–421.

LOEB, LEO, 1897 Über Transplantation von weisser Haut auf einem Defekt in schwarzer Haut und umgekehrt am Ohr der Meerschweinchens. 1915. Arch. f. Entwicklungsmech. Organ. **6**: 1–144.

MOORE, A. R., 1910 A biochemical conception of dominance. Univ. of California Publ. Phys. **4**: 9–15.

MORGAN, T. H., 1922 On the mechanism of heredity. Croonian lecture. Proc. Roy. Soc.London B. **94**: 162–197.

1926 The theory of the gene. 343 pp. New Haven: Yale University Press.

ONSLOW, H., 1915 A contribution to our knowledge of the chemistry of coat colors in animals and of dominant and recessive whiteness. Proc. Roy. Soc. London B. **89**: 36–.

ONSLOW, M. W., 1923 Oxidizing enzymes VI. A note on tyrosinase. Biochem. Jour. **17**: 216–219.

PRZIBRAM, H., 1924 Die Rolle der Dopa in den Kokonen gewisser Nachtfalten und Blattwespen mit Bemerkungen über die chemischen Orte der Melaninbildung. Arch. f. Mikr. Anat. u. Entwicklungsmech. **102**: 621–634.

PRZIBRAM, H., DEMBOWSKI and BRECHER, L., 1921 Einwirkung der Tyrosinase auf "Dopa." Arch. f. Entwicklungsmech. Organ. **218**: 140–165.

RÉNYI, G. S., 1924 Studies on pigment genesis. I. The nature of the so-called "Pigmentbildner." Jour. Morphol. **39**: 415–433.

SATO, K., and BRECHER, L., 1925 Kann Dopa oder Tyrosin das Chromogen bei Wirbeltieren abgeben? Arch. f. Mikr. Anat. u. Entwicklungsmech. **104**: 649–658.

SCHULTZ, W., 1918 Versteckte Erbfactoren der Albino für Färbung beim Russenkaninchen im Soma dargestellt und rein somatisch zur Wirkung Gebracht. Zeitschr. indukt. Abstamm. u. Vererb. **20**: 27–40.

1923 Erzeugung der Winterschwarz. Willkürlich Schwarzung gelber Haare. Arch. f. Entwicklungsmech. Organ. **51**: 338–382.

1925 Verhalten der einzelnen Färbungsgene zur Dopa reaktion bei Kaninchenrassen. Arch. f. Entwicklungsmech. Organ. **105**: 677–710.

TROLLAND, L. T., 1917 Biological enigmas and the theory of enzyme action. Amer. Nat. **51**: 321–350.

WRIGHT, SEWALL, 1916 An intensive study of the inheritance of color and of other coat characters in guinea pigs with especial reference to graded variations. Carnegie Inst. Washington. Publ. 241, Part II, pp. 59–121.

1917 Color inheritance in mammals. Jour. Heredity **8**: 224–235.

1920 The relative importance of heredity and environment in determining the piebald pattern of guinea pigs. Proc. Nat. Acad. Sciences. **6**: 320–332.

1923 Two new color factors of the guinea pig. Amer. Nat. **57**: 42–51.

1925 The factors of the albino series of guinea pigs and their effects on black and yellow pigmentation. Genetics **10**: 223–260.

1926 Effects of age of parents on characteristics of the guinea pig. Amer. Nat. **60**: 552–559.

WRIGHT, SEWALL, and EATON, O. N., 1926 Mutational mosaic coat patterns of the guinea pig. Genetics **11**: 333–351.

STUDIES IN THE CAUSES OF DENTAL ANOMALIES

W. G. DOWNS, JR.

Department of Anatomy, Indiana University, Bloomington, Indiana

Received May 20, 1927

In the studies basic to this paper, a large number of cases was included from the SOUTHERN INDIANA HOSPITAL FOR THE INSANE at Evansville, the INDIANA SCHOOL FOR FEEBLE-MINDED YOUTH at Fort Wayne, and the private practices of the author and his associates. Careful records were kept of both the dental and systemic findings for all of the patients, and where possible, records were obtained of the families of these patients. In the case of the School for the Feeble-Minded, in particular, these records were extremely complete and accurate, some of them extending back to the date of organization of the present institution—nearly thirty years ago; and many of them covering a period of ten years. A careful review of the literature, in its dental, endocrine and genetic phases, was made. Experiments were made with feeding and injecting dogs with the extracts of both the anterior and posterior lobes of the pituitary gland. Finally, experiments in feeding the extracts of both lobes of the pituitary gland are still in progress on a group of selected cases in the INDIANA SCHOOL FOR FEEBLE-MINDED YOUTH.

Six hundred and forty-seven clinical cases were studied. These were divided in accord with the endocrine diagnoses, as follows: cretins, mongols, dwarfs, giants, acromegalics, polyglandular dyscrasias, hermaphrodites, border-line cases, adiposo-genitalias, diplopias, goitres, microcephalics, macrocephalics, virilisms, infantilisms, myxoedemas and normals. The same group of cases was then divided according to the dental anomalies found, as follows: missing laterals, other teeth congenitally missing, peg-shaped laterals, super-numeraries, spaced uppers, bunched lowers, prognathisms, mesognathisms, and normals. The two classifications were then cross-consolidated to form table 1.

The purpose of this tabulation was to determine if certain types of dental anomalies occurred regularly in definite types of endocrine dyscrasias. It was felt that in order to determine this occurrence, either a certain type of dental anomaly must be found most frequently in cases of a certain type of endocrine dyscrasia, or that a majority of the cases of a

TABLE 1

GLANDULAR DYSCRASIAS	NORMALS (APPROXIMATELY)	MISSING LATERALS	OTHERS MISSING	PEG-SHAPED LATERALS	SUPERNUMERARIES	SPACED UPPERS	CROWDED LOWERS	PROGNATHISM	MESOGNATHISM	TOTALS
Cretins	17	4	0	2	0	1	2	2	1	29
Mongols	19	4	3	1	0	3	4	1	2	37
Dwarfs	5	1	1	1	2	3	3	2	1	19
Giants	5	0	1	1	2	5	3	5	1	22
Acromegalics	11	1	0	1	1	4	2	4	1	25
Polyglandulars	20	3	3	1	2	7	8	4	2	50
Hermaphrodites	4	1	0	1	0	1	2	0	1	10
Borderlines	41	4	3	2	2	12	17	7	4	92
Adiposo-geni.	3	1	1	1	0	2	2	0	1	11
Diplopias	6	0	0	1	1	2	3	0	1	14
Goitres	16	1	2	1	2	2	5	1	0	30
Microcephalics	1	1	0	0	0	0	1	0	1	4
Macrocephalics	2	0	0	0	1	1	0	1	0	5
Infantilisms	2	1	1	0	0	0	0	0	1	5
Virilisms	3	0	0	1	1	0	2	1	0	8
Myxoedemas	3	1	2	0	1	3	0	3	1	14
Normals	229	4	1	3	1	7	18	6	2	271
Totals	387	27	18	17	16	53	71	37	20	647

Explanation—Figures above refer to cases studied, the horizontal ones being the endocrine groups, divided vertically to demonstrate how these in turn fall into the groups of dental anomalies. Cases were assigned to definite endocrine groups, only if the clinical picture presented was clear-cut. If there was any grave doubt as to the proper grouping, they were classed as "borderline" or "polyglandular" cases. It was not attempted to group the various kinds of "goitre" cases. Very probably all of the cases listed as "giants" are not true pathological giants, nor are all of those classed as hermaphrodites true "mixed" sex cases. It may be readily seen that a definite diagnosis in such cases would require autopsy or at least operative procedure. Dentally, cases were only classed as "spaced," "bunched," "prognathic," etc., if it could be reasonably assured that the condition was not due to operative or traumatic causes. On the whole, it is felt that the above classification is as accurate as available clinical diagnostic methods could make it.

definite dyscrasia must likewise present a particular type of anomaly. Such was not found to be the case.

Referring to table 1, it will be seen that in no case did there appear to be any typical coincidence of endocrine dyscrasia and dental anomaly. Missing laterals, peg-shaped laterals, crowded lower anteriors, cases of prognathism and the other anomalies all occurred with about equal frequency in the various endocrine dyscrasia types. It was found, however, that close to fifty percent of all of the cases of endocrine dyscrasias likewise had dental anomalies of one or the other types, while the larger group of normal cases showed only about seventeen percent of dental anomalies. Thus, while it was evident that dental anomalies are more frequent in endocrine cases, no conclusion of a positive nature could be drawn regard-

ing the regularity of occurrence of dental anomalies of a definite type in specific endocrine dyscrasias. It would, then, be difficult to understand how the appearance of a dental anomaly could be considered as pathognomonic of a particular type of endocrine dyscrasia.

While the literature in the endocrine, dental, and genetic fields bearing on the causation of dental anomalies is relatively scarce, its review was nevertheless productive of the finding of widely variant results and views. On the whole, its trend was toward a particular endocrine causation of specific dental abnormalities. KAPLAN (1917), WHEELON (1925), ENGELBACH and TIERNEY (1922), CEDERCREUTZ (1925), WARD (1925), TIMME (1918), PETZEL (1922), KRANZ (1912), GRAVES (1904), and HOWARD (1926) seemed to lean in varying degrees toward this view. HOWARD (1926) reports over six hundred cases studied and obtained results not coinciding with those included in this study. This was especially true in regard to the incidence of missing teeth in mongols. Inasmuch as it is extremely difficult to obtain accurate records of previous dental operations in patients of this type, it is at least possible that the findings in some of his cases were due to earlier extractions. In most of the cases reported in the literature, it is felt that there has not been a sufficiently large series of cases examined from this angle, or that the cases have not been carefully enough controlled to make the findings valid.

On the other hand, some workers have cited about an equal number and type of cases supporting the genetic origin of dental anomalies, rather than the endocrine. Among these latter are: LUCAS (1888), KEITH (1919), BEADLE (1926), PITTS (1922), THADANI (1921), and HOPSON (1920), while CASTLE (1916) is convinced that factors influencing dental development, like those concerned with the length and color of hair are undoubtedly genetic, and MAUD SLYE (1926) asserts that all regular types of variations from normal in any of the organs have a genetic origin.

In the feeding experiments, five litters of four puppies each were used, the average age for the entire series at the beginning of the experiments being four weeks, none being over six weeks of age. The animals were all about the same size, averaging about 2 kg in weight at the beginning of the experiments. No regard was paid to sex in these experiments[1]. In each litter, one dog was fed the extract of the anterior lobe, one that of the posterior lobe, one that of the whole gland, and one, the control animal, was fed a normal diet only. Armour's tablets made from the dried ox-gland were used throughout. The dosage was computed so as to be approximately proportionate to the maximum dosage for a

[1] The animals used throughout were mongrels, so that although the blood was the same in all of those within a litter, the litters were not known to be homozygous for any character.

human of average weight, which was, in the case of the anterior lobe extract, .035 gm, of the posterior lobe extract, .0016 gm, and of the whole gland extract, .035 gm. The extract was administered regularly three times a day, each animal was given 4 gm of codliver oil (Mead-Johnson Company's) once a day, to offset any possibility of rickets, and all of the animals were wormed, when the need for it became evident.

Beginning four weeks from the start of the experiment, entire litters were killed at intervals of four weeks. Tissues were fixed in 10 percent formalin, decalcified in 5 percent HCl in 67 percent ethyl alcohol, and paraffin sections were made through the upper and lower maxillae at such an angle as to include the temporary teeth and the developing permanent ones.[2] These sections were then stained with chloral hemotoxylin and counterstained with eosin. The sections were carefully studied and compared, but no definite variations in placement, form or texture could be noted in either temporary or developing permanent teeth.

For the injection experiments, dogs likewise were used. The age of the animals was the same as that of those in the preceding experiments. Six litters of three puppies each were used. In each litter, during the first six weeks, one dog was injected with .18 cc of antuitrin (Parke, Davis and Company) twice daily, one dog received .18 cc of pituitrin (Parke, Davis and Company) twice daily, while one, the control animal, received a like amount of sterile water. Although no apparent untoward local or systemic effects were observed from this rather excessive dosage, six weeks after the beginning of the experiment, it was felt better to use a more nearly proportionate dosage and the amount was reduced to .06 cc twice daily, in each case.

As in the preceding series, litters were killed at intervals of four weeks from the beginning of the experiments, except in the case of the last litter, in which the death from spasms, of the animal receiving anterior lobe extract made it necessary to kill the remaining members of the litter two weeks earlier than had been contemplated. Sections were made of the maxillae of all of these animals and prepared in the same manner as in the preceding series.

While some very interesting and surprising systemic results were obtained, which will be discussed in a later paper, no definite changes in the placement, form or texture of the temporary or the unerupted permanent teeth could be demonstrated.

In none of the cases, in either the feeding or injecting experiments, were

[2] It was felt to be better to study the effect on the permanent dentition prior to eruption, as decalcification of an erupted tooth destroys the enamel completely, whereas if decalcified in its crypt, all of the structures remain intact.

there any apparent histological differences in the number, form, size or nuclei of the ameloblasts; the size, arrangement or form of the forming enamel rods; the number, size, arrangement or form of the dentinal tubules; or the number, size, form or nuclei of the odontoblasts or of the various pulpal cells. Neither was there any apparent interference in the form, size or placement of the erupted temporary teeth or of the unerupted permanent teeth. In short, neither type of medication, simulating in some degree over-secretions of the gland, seemed to influence to any extent the teeth of these animals.

In seven months of administration of the pituitary extracts, per orum, to the patients of the INDIANA SCHOOL FOR FEEBLE-MINDED YOUTH, no evidence of any effect on the dental mechanism has been noted. Considering the length of time required for the formation of the human dentition, no results could be expected in such a period. These experiments are still in progress.

From the above-detailed series of experimental studies, it is felt that certain tentative conclusions may be warranted. While the endocrines may, and judging from the literature, probably do, play an important part in calcium metabolism, they do not apparently act in such a way as to cause regular types of dental anomalies (see table 1).

In the cases studied clinically, it was noted how frequently the anomalies were found recurring in families. For this reason, a study was made of the possibility of the occurrence of a genetic factor in the production of dental anomalies. Seventy-one patients showing very typical anomalies were selected for this study, and the group was divided in accordance with

TABLE 2

DENTAL ANOMALIES	FAMILY HISTORIES OBTAINED		FAMILY HISTORIES NOT OBTAINABLE	TOTALS OF EACH TYPE
	Histories Positive	Histories Negative		
Missing Laterals	9	0	2	11
Others Missing	2	0	0	2
Peg-shaped Laterals	2	1	2	5
Supernumeraries	2	1	3	6
Spaced Uppers	10	1	5	16
Crowded Lowers	9	1	8	18
Prognathism	3	1	1	5
Mesognathism	3	1	1	5
Totals	40	6	22	68

Explanation—While the above figures refer to individuals studied, columns 1 and 2 likewise represent families, as where the history was obtained, the entire family was charted and studied. The series is divided into the different types of anomalies, only as a matter of secondary interest, to demonstrate the proportions found.

the type of anomalies presented. Of these seventy-one cases, forty-seven fairly complete family dental records could be obtained.

In twenty-four cases no family dental record was obtainable. Of the forty-seven cases in which a fairly complete family record was obtained, five showed no dental anomaly in other members of the family, while forty-two did show one or more other anomalous cases of a similar character in the family. The forty-two families showing positive results were then studied to determine what proportion of anomalies was found in each generation (see table 3).

TABLE 3

	TOTAL NUMBER INDIVIDUALS PER GENERATION	TOTAL NUMBER ANOMALOUS INDIVIDUALS PER GENERATION	TOTAL NUMBER NORMAL INDIVIDUALS PER GENERATION	RATIO ANOMALOUS INDIVIDUALS TO NORMAL INDIVIDUALS
Parental Generation	84	39	45	3.4:4
Filial 1 Generation	177	21	156	0.5:4
Filial 2 Generation	382	107	275	1.5:4
Filial 3 Generation	219	64	155	1.6:4
Filial 4 Generation	16	8	8	40 :4

Explanation—This table was made by taking the entire number of cases of each type from the totals in each generation of the forty-two positive cases for which histories were obtainable, from column 1 of table 2. Thus, the ratios are cross-sections of each generation of the entire forty-two families. There is a regularity which bespeaks adherence to some genetic system.

It was felt that the constant recurrence of a certain type of anomaly within a given family, offered of itself, considerable evidence of a genetic causative factor. If, however, in addition to this, some regular formulae or ratios, describing these occurrences could be found, it would certainly lend strength to the original evidence offered. In determining these ratios, the first generation obtainable in each family was classed as the Parental, the next as the Filial 1, etc. The total numbers of individuals through each complete generation of the entire forty-two families was then divided into those showing a familial anomaly and those not showing it, classed as normals. The results of this tabulation (see table 3) showed eighty-four individuals in the Parental generation, of which thirty-nine had the anomaly which was found appearing in their respective families, while forty-five showed no evidence of it. This gave, in the Parental generation, a ratio of 3.4:4.0. In the next, or Filial 1 generation, of a total

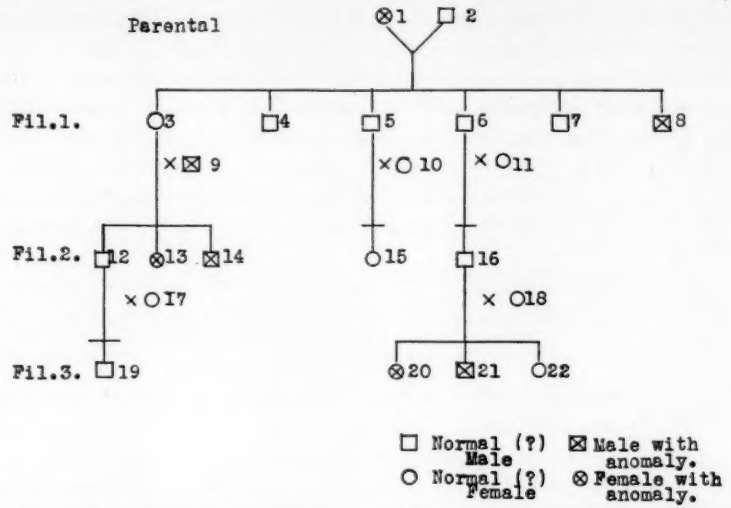


FIGURE 1.—Explanation—Figures containing an x (⊗ 9, etc.) represent individuals having the anomaly—in this case, a very distinctive type of marked prognathism. Plain figures merely do not show it or at least, not characteristically. It is probable that those who do show it are homozygous recessives, but it is difficult to determine the genetic character of these who do not show it.

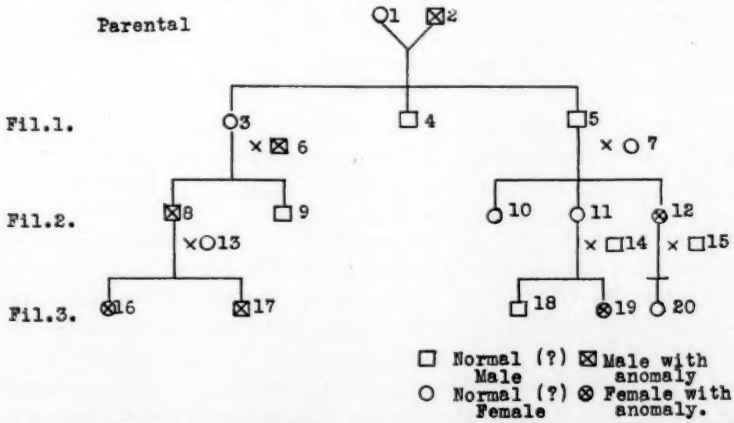


FIGURE 2.—Explanation—Explanation of figure 1 likewise applies to this, except that the anomaly in this case consists of missing laterals. No individual in this chart including the collateral lines, has laterals sufficiently large and well-formed to be considered fully normal, which, of course, makes it more difficult of even a tentative interpretation.

of one hundred and seventy-seven individuals, twenty-one exhibited the familial anomaly, while one hundred and fifty-six did not. The ratio yielded in this case, then, was .5:4.0. The Filial 2 generation, having a total of three hundred and eighty-two individuals, had one hundred and seven with their respective familial anomalies and two hundred and seventy-five without it. The ratio in this case, then, was 1.5:4.0. A total of two hundred and nineteen individuals of the Filial 3 generation disclosed sixty-four having the anomaly as against one hundred and fifty-five not having it, showing a slightly augmented ratio of 1.6:4.0. It was only possible to obtain Filial 4 family records in five cases, which yielded a total of sixteen individuals of this generation, of which exactly one-half showed the family anomaly and one-half did not, resulting in a balanced ratio of 1.0:1.0.

Where positively complete family dental records were obtainable, in a few instances the families were genetically charted as in figures 1 and 2.

Hypothetical genetic characters were given to the various individuals in the family, temporary charts were constructed, were corrected, new hypotheses were attempted and again corrected, until apparently probable schema were obtained. Figures 1 and 2 show the results in two of these cases. In one especially noticeable case, which is in the same family as that shown in figure 1, the anomalous individuals showed such exactly similar conditions in the different generations that one case was selected from each living generation, casts made, and photographs of these casts were taken. (Figures 3 to 5.)

While the results of the genetic tabulations do not yield Mendelian ratios, it will be remembered that in dealing with human genetics, so many factors enter into a given result that the probable error is enormous. Considering this, there is a regularity in the ratios in the sequence shown, which bespeaks adherence to some sort of a genetic system. It is probable, but undetermined, that the dental anomalies are simple recessive characters (CASTLE 1916). In any event, the appearance of these anomalies within given families is so regular that the hereditary influence seems more than likely.

In summary, the conclusions from the above-detailed series of investigations are fairly obvious. Although it is possible that dental anomalies may sometimes appear as the result of endocrine dyscrasias, just as may be the case with vitamine deficiencies or other developmental interference, it is very unlikely that there is any regularity about such appearance.

Dental anomalies probably cannot be considered as pathognomonic of any definite type of endocrine dyscrasia.

It would seem to be fairly certain that anomalies in dentition are recurrent in families as the result of the operation of genetic or hereditary factors.

FIGURE 3.—Cast of the mouth of individual 8 from figure 1 (Generation F1, Age 53).

FIGURE 4.—Cast of the mouth of individual 14 from figure 1 (Generation F2, Age 39).

I am greatly indebted for the assistance of Dr. J. A. BADERTSCHER, of the Department of Anatomy of the INDIANA UNIVERSITY SCHOOL OF MEDICINE for the oversight of my work; to Dr. L. P. HARSHMANN of the

INDIANA SCHOOL FOR FEEBLE-MINDED YOUTH for extending the facilities of that institution and for much valuable assistance in diagnosing and handling cases; and to Dr. W. G. DOWNS, SR., of Evansville, and Drs.

FIGURE 5.—Cast of the mouth of individual 20 from figure 1 (Generation F3, Age 6—temporary teeth). Note the regularity in type of these three cases. Other individuals from this family show, as nearly as is possible, an identical condition.

BEALE, RAWLES, HILGEMANN and DUEMLING of Ft. Wayne, Indiana, for placing at my disposal a great deal of valuable clinical and bibliographic material.

LITERATURE CITED

BANDLER, S. W., 1920 The endocrines. 58 pp. Philadelphia: W. B. Saunders Co.

BEADLE, O. A., 1926 An inherited anomaly of dentition. Jour. Genetics **17**: 72–89.

BELL, W. BLAIR, 1918 The pituitary. 94 pp. New York: Wm. Wood & Co.

CASTLE, W. E., 1916 Genetics and eugenics. 241 pp. Cambridge: Harvard University Press.

CEDERCREUTZ, A., 1925 Sind innersekretorische Störungen auf kongenital-luetischer Grundlage als Ursache der hutchinsonischer Triad? Münch. Med. Woch. **72**: 1960–1962.

CUSHING, H. C., 1912 The pituitary body and its disorders. 331 pp. Philadelphia: J. B. Lippincott and Co.

ENGELBACH, W., 1920 Classification of disorders of the hypophysis. Endocrinology **4**: 347–365.

ENGELBACH, W., and TIERNEY, J., 1922 Tice's practice of medicine. Vol. **8**: 5: 337–738.

GARRISON, F. H., 1922 Endocrinology and metabolism. Vol. **1**: 45–80. New York: D. Appleton and Co.

GRAVES, W. W., 1904 Ueber Lückenbildung zwischen den einzelnen zähnen; ein fruhdiagnostiche, und bisher wenig bekannter zeichen der Akromegalie. Monat. F. Psych. u. Neur. **16**: 18–48.

HALSEY, J. T., 1922 Endocrinology and metabolism. Vol. 1: 81–152. New York: D. Appleton and Co.

HOPSON, M. F., 1920 Hemihyperplasia of face, jaws, and teeth. Proc. Roy. Soc. Med. Sect. Odont. Pt. III, 13: 67–70.

1921 Complete absence of deciduous and permanent teeth. Proc. Roy. Soc. Med. Sect. Odont. Pt. III, 14: 23–26.

HOSKINS, R. G., 1922 Endocrinology and metabolism. Vol. 2: 919–930. New York: D. Appleton and Co.

HOWARD, C. C., 1926 A study of jaw and arch development considered with the normal and abnormal skeleton. Jour. Ortho. Oral Surg. and Rad. 12: 3–16.

KAPLAN, D. M., 1917 An endocrine interpretation of the dental apparatus. Endocrinology 1: 208–221.

KEITH, A., 1919 The differentiation of mankind into racial types. Lancet. 2: 552–556.

KRANZ, P., 1912 Schilddruse und Zähne. Deut. Monat. F. Zahnheil. 30: 1–35.

LEWIS, DEAN, 1922 Endocrinology and metabolism. Vol. 1: 721–736. New York: D. Appleton and Co.

LUCAS, H. C. 1888 On the congenital absence of an upper lateral incisor tooth as a forerunner of a hair-lip and cleft-palate. Trans. Clin. Soc. 21: 64-66

NOVAK, E., 1922 Endocrinology and metabolism. Vol. 1: 153–160. New York: D. Appleton and Co.

PETZEL, W., 1922 Die endrokrinen Drüsen in ihrer Beziehung zu Zahn und Kieferanomalien. Deut. Med. Woch. 48: 231.

PITTS, A. T., 1921 Some cases of hypoplasia of deciduous dentition. Proc. Roy. Soc. Med. Sect. Odont. Pt. III, 14: 28–32.

1923 Absence of the permanent teeth with normal absorption of their deciduous predecessors. Proc. Roy. Soc. Med. Sect. Odont. Pt. III, 14: 33.

POTTENGER, F. M., 1922 Endocrinology and metabolism. Vol. 1: 161–180. New York: D. Appleton and Co.

SHARP, J. G., 1916 Atrophy of the thyroid gland with hypertrophy of the pituitary gland. Clin. Jour. 45: 290–291.

SIMONDS, J. P., 1922 Endocrinology and metabolism. Vol. 1: 767–804. New York: D. Appleton and Co.

SLYE, MAUD, 1926 The hereditary character of cancer. Jour. of Canc. Res. 10: 15–49.

THADANI, K. J., 1921 A toothless type of man; The Bhuddas of India; A case of sex-linked inheritance. Jour. Heredity 12: 87–88.

TIMME, W., 1918 A new pluriglandular compensatory syndrome. Endocrinology 2: 209–240.

UHLENBUTH, E., 1921 Experimental gigantism produced by feeding pituitary gland. Proc. Soc. Exp. Biol. and Med. 18: 11–14.

1921 Experimental production of gigantism by feeding the anterior lobe of the hypophysis. Jour. Gen. Physiol. 3: 347–365.

WARD, W. G., 1925 The relation of the endocrine glands to the teeth in rickets. Med. Jour. and Rec. 122: 743–744.

WHEELON, H., 1925 Clinical significance of the congenital absence of the upper lateral incisors. Endocrinology 9: 35–59.

INDEX

Prepared by DOCTOR CONWAY ZIRKLE, graduate student, BUSSEY INSTITUTION. Page numbers printed in black-faced type refer to headings of articles or sections. Numbers in italics indicate reference in lists of "Literature Cited."

ABEGG, F. A. 484
DE ABERLE, S. B. **340**, 344, *347*
AIDA, TATUO 254, 276, 277, *278*
ALLEN, C. E. 60
ALLEN, I. M. 449, 454, *460*
ALTENBURG, E. *165*
Amarantus paniculatus **242**, 252
  Genetic types of "striped form" 244
  Mosaic-eared individuals **248**
  Red-eared individuals **244**
  Variability of a white-eared form **242**
Ameba radiosa 261
ANDERSON, E. G. *306*, 447, *447*
ANDERSON, E. S. 397
Antirrhinum 408, 447
Aplocheilus 254, 276
Apogamy (Fragaria) 322
Aquilegia 447
Axolotl 265
BADE, E. 261, *278*
BADERTSCHER, J. A. 578
BANDLER, S. W. *579*
BARANOV, P. 64, 65, *82*
BARLOW, N. 395, 396, 397, 398, 401, 405, 406, 408, *414*
BATESON, W. 395, 396, *414*
BAUMBERGER, J. P. 48, *57*
BAUR, E. 447, *447*
BEADLE, O. A. 572, *579*
BEALE, 579
BEAN, B. A. 254, *278*
Bean
  Brockton Pole 117, 123
  Horticultural Pole 117, 123
  Improved Goddard 117, 123
  Kentucky Wonder 117, 123
  Lyon 116
  Marblehead 117, 118, 119, 120, 121, 122, 123
  Navy Pea 117, 123
  Powell Prolific 117. 118, 119, 120, 121, 122, 123
  Seed, The immediate effect of cross-pollination on size and shape of **115**
  Tennessee Greenpod 117, 123
  Velvet 116
  Yellow Eye, 117, 123
BELL, W. B. *579*
BELLAMY, A. W. 254, 259, 266, 276, 277, *278*
BELLING, J. 74, 79, *82*, 84, 85, 90, *92*, 116, *123*, 295, *306*
Belling's iron-aceto-carmine 450
BLACKBURN, K. B. 90, *91*
BLAKESLEE, A. F. 74, 79, *82*, 84, 85, 90, *91*, 92, 295, *306*
BLISS, I. C. 46, 47, *57*
BLOCH, B. 553, *567*
BÖNICKE, L. V. 84, 92
BONNIER, G. 12, 24, *57*
BOULENGER, 259, 262
BOVERI, TH. 336, *338*
BRECHER, L. 553, *567*, *568*
BRIDGES, C. B. 2, 3, 6, 9, 12, 14, 25, 28, 29, 30, 35, 36, 54, *57*, *58*, *165*, 295, *306*, 389
BRIEGER, F. G. 397
BRIND, W. L. 261, *278*
BRINK, R. A. **348**, *348*, 397, **461**, 466, 468, **471**, 485, 486, 487, 488, *491*
BRUNSON, A. M. 416, *439*, *504*
BURLISON, W. L. *439*
BURNHAM, C. R. **348**, **461**, 471, 484, 485, *491*
CARNOT, P. 554, *567*
CAROTHERS, E. E. 389, *392*
CARRIER, L. 116, *123*
Case of Non-disjunction in the Mouse, **295**
CASTLE, W. E. 277, *278*, 531, 535, *567*, 572, 577, *579*
Cavia cutleri (see guinea pig) 530
Cavia rufescens 531
CEDERCREUTZ, A. 572, *579*
Chaenomales 324
CHEN, 51
Chlorophyll
  Yellow green chlorophyll in maize, **492**
Chlorophyll deficiencies in maize, A genetic study of **415**
Chromosome aberrations, Effect on *Drosophila melanogaster* **1**
Chromosome constitution of Gates' "non-disjunction" (*v–o*) mice 379
Chromosome counts in the varieties of *Solanum tuberosum* and allied wild species **84**
Chromosome shape in Matthiola, Mendelian inheritance of **449**
Chromosomes, individuality of, **64**
  in *Ranunculus acris* L. **66**
CLARK, C. F. 85, 91, *92*
CLARK, H. W. 254, *278*
Cockscomb 244
COLE, L. J. 416
COLLINS, G. N. 115, 122, *124*, 337, *338*, *447*, 524, *529*
Color-factors of the guinea pig **530**,
Color wheel 530
CONKLIN, E. G. *347*
CORRENS, C. 244, *252*, 333, *338*, 395, *414*, 447, *447*
Cotyledon color, Inheritance studies in soybeans **441**

Cox, E. K. 380, 391, *392*
Crategi 73
Crategus smithii 79
Crepis capillaris 65
Crew, F. A. E. 277, *278*
Cushing, H. C. 579
Cypriandotes, 256, 258, 276, 277, 278
Cytological and morphological investigations of gynodimorphic and normal forms of *Ranunculus acris* L. **59**
Dahlgren, K. V. O. 395, *414*
Danforth, C. H. **340**, 341, *347*
Darlington, C. D. 65, 66, *82*
Darrow, G. M. 308
Darwin, 393, 395, 397, 408
Datura 74
Datura stramonium 84, 90
Datura tatula 84
Davenport, C. B. *278*, 533, *567*
Deflandre, Cl. 554, *561*
Delage, Y. 336, *338*
Delaunay, L. 66, 82
Dembowski, 553, *568*
Demerec, M. 417, 438, *439*, 494, *504*
Dental anomalies, Studies in the causes of **570**
Detlefsen, J. A. 344, *347*, 531, *567*
Dexter, J. S. 2, 50, *57*
Differential action, sugary gene in maize **348**
Domin, K. 60, 79, *82*
Downs, W. G. 579
Downs, W. G., Jr. **570**
Drimiopsis maculata 64
Drosophila funebris, **128**, 129, 130, 153, 163
  Alae divergentes **153**
  Phenotypic manifestation of the genovariation radius incompletus **128**
  Phenotypic manifestation of genotype **161**
  Phenotypic manifestation of hereditary characters **129**
  Radius incompletus 125, 128
    Appearance and selection **133**
    Crossing various lines **147**
    Different "strength" of manifestation in different lines **156**
    Effects of selection in pure and heterogeneous lines **147**
    Inhibitor **149**
    Recessiveness and dominance **159**
  Reduction of genetalia in males **154**
    Selection of "feeble" and "strong" lines **144**
    Sex-limitation **159**
    Single genes effecting expression of **149**
  Reverse genovariation in **125**
  Venae transversae incompletus **155**
Drosophila melanogaster 129, 130, 163
  Aberrations that involve a whole chromosome **25**
  Autosomal deficiencies **5**
  Chromosome aberrations **40**
  Correlation, length of larval and pupal period **46**
  Deficiency and duplication of the fourth chromosome **28**
  Deficiency and duplication of the X chromosome **26**
  Double yellow **27**
  Effect of chromosome aberrations **1**
  Haplo IV **28**
  Haplo IV flies, Retarded stages in **42**
  Minute **9**
  Notch deficiencies **2**
  Notch male **3**, 41
  Nullo-IV 40
  Nullo-X 40
  Pale duplication 41
  Pale translocation **12**
  Plexate **6**
  Sectional aberrations **24**
  Sectional duplications **12**
  Triplo-IV **35**, 41
  Triplo-X 41
Duchesnia indica 311, 321, 324, 325, 338
Duemling, 579
Duncan, G. H. *439*
Dunn, L. C. 109, 110, *114*
Durham, F. M. 552, *567*
Durham, G. B. *294*
Dvorak, Lila 253
East, E. M. **307**, **393**
Eaton, O. N. 554, *569*
Effect of chromosome aberrations on development in *Drosophila melanogaster* **1**
Effects in combination of the major color-factors of the guinea pig **530**
Elderton, W. P. 416, *439*
Elodea 266
Embody, G. C. 253
Emerson, R. A. 117, 244, *252*, 349, *378*, 416, 431, *439*, 495, *504*
Emme, H. 65, *82*
Engelbach, W. 572, *579*
Ernst, A. 80, *82*, 395, *414*
Euchlaena, 337
Evermann, B. W. 254, *278*
Exachorda 324
Eyster, W. H. 421, *439*
Factor for yellow-green chlorophyll color in maize and its linkage relations **492**
Fagopyrum 395
Farnum, M. E. 90, *92*
Feustel, R. M. 570
Finck, M. C. 256, 259, *278*
Focke, W. O. 315, *338*
Fragaria
  The 7-chromosome group **308**
  The 21-chromosome group **310**
  The 28-chromosome group **310**
  7-chromosome species × 21-chromosome species **314**
  7-chromosome species × 28-chromosome species **315**
  28-chromosome species × 21-chromosome species **320**
  28-chromosome species crossed *inter se* **322**
Fragaria americana alba 309, 313, 314, 316, 326
  Ananas 335
  Belle Bordelaise 333
  Black Hautbois 310, 332, 333, 334
  bracteata 309, 313, 314, 318
  californica 309, 313
  chiloensis 310, 315, 322, 323, 329, 331

chili velu 333
cuneifolia 310
Docteur Nicaise 333, 334
elatior 310, 314, 315, 320, 332, 333, 334, 338
Fraiser des Alpes 333
Fraiser des quatre saison blanc 332
Gaillon rouge 333
Generic crosses **321**
glauca 310, 316, 317, 319, 322
Globe 335
grandiflora 311
Haverland 323
Kellogg's Prize 323
Mexicana 309, 313, 314
Millardet's "faux hybrides," **329**
Monstreuse Hautbois 310
moschata 310
Parthenocarpy, parthenogenesis and apogamy **322**
Sample 323
Studies on the genetics of **307**
Technique used in making crosses **311**
vesca 308, 309, 310, 314, 315, 317, 321, 322, **325**, 329, 332, 333, 334, 336, 338
Inheritance of flour color and fruit color **325**
vesca rosea 313, 314, 317, 318, 325
virginiana 308, 310, 317, 318, 319, 320, 322, 323, 334, 336
Warfield 323
FRASER, A. C. 253
FROST, H. B. **449**, *460*
FULTON, R. 106, *107*
Functional inter-relation of certain genes in the development of the mouse **340**
FÜRTH, 553
Galtonia candicans 64, 66
Gametophyte (see maize)
GARRISON, F. H. *579*
GATES, R. R. 90, *92*, 295, *306*
GATES, WM H. **295**, 297, 301, *306*, 379, 380, 383, 384, 387, 388, 389, 391, 392, *392*
Genetic analysis of white margined flowers in the Japanese morning-glory **199**
Generic crosses (Fragaria) **321**
Genetic study of certain chlorophyll deficiencies in maize **415**
Genetics of Fragaria, Studies on the **307**
Genetics of a viviparous top-minnow Platypoecilus: the inheritance of two kinds of melanophores **253**
Genovariation, A reverse, in *Drosophila funebris* **125**
Phenotypic manifestation of **128**
GERLACH, G. 256, 259, *278*
Germ plasm of *Phaseolus vulgaris*, Some attempts to modify **284**
GERSCHLER, M. W. 266, *278*
GIARD, A. 336, *338*
GILBERT, C. H. *279*
GILL, T. 254, *278*
GIRARD, C. 254, 256, *278*
GOLDSCHMIDT, R. 552, *567*
GONZALEZ, B. M. 49, *57*, *58*
GORDON, MYRON **253**, 256, 259, 261, 265, *278*
GORTNER, R. A. 553, 555, 567
GOWEN, 2
GRAVES, W. W. 572, *579*
GREGORY, R. P. 395, *414*, 447, *447*
Green Mountain 90
Guinea pig
Absence of linkage **547**
Color factors 530
Combination of factors *f* and *b* **543**
Combination of factors *f* and *p* **544**
Combinations of the albino series with factors *b* and *P* **535**
Dilution factor *f* **538**
Effects in combination of the major color-factors **530**
Environmental effects **547**
Grades on color wheel **533**
Grades of colors **532**
Imperfect dominance of *f* **546**
Peruvian cavy 530
GÜNTHER, H. 254, 256, 259, 261, *279*
Gynodimorphic and normal forms of *Ranunculus acris* L., Cytological and morphological investigations **59**
HAASE-BESSELL, C. 80, *82*
HABERLANDT, G. 323, *338*
HAECKER, V. 158, 164, *165*
HAGIWARA, TOKIO, 199, 221, 240, *241*
HALSEY, J. T. *580*
HALSTED, B. D. 116, *124*
HARPER, R. A. 60
HARRIS, J. A. 60, 285, *294*, 416, *439*
HARRIS, R. G. 253
HARRISON, J. W. H. 90, *91*
HARSHMANN, L. P. 578
HASHIMATO, K. 239
Heterostyly in Lythrum salicaria, The inheritance of **393**
HILDEBRAND, 393, 396
HILGEMANN, 579
HOCQUETTE, M. 80, 81, *82*
HOFMANN, FREDERICK WENZL, **284**
HOGE, M. A. 50, *57*
HOHLMANN, K. *279*
HOLBERT, J. R. 438, *439*
HOOKER, D. 554, *568*
HOPSON, M. F. 572, *580*
HOSHINO, 525
HOSKINS, R. G. *580*
HOTTES, C. F. 284, *294*
HOWARD, C. C. 572, *580*
HUBBS, C. L. 254, 256, *279*
HUTCHISON, C. B. 494, 504, *504*
IBSEN, H. I. 341, *347*, 531, *568*
ICHIJIMA, K. 308, 331, 336, *338*
ILJIN, N. A. 550, *568*
Illegitimate unions (Lythrum), 394
IMAI, YOSHITAKA, **199**, 200, 215, 240, *241*, **242**, 244, 252
Immediate effect of cross pollination on the size and shape of bean seed **115**
IMMS, A. D. 51, *57*
Inheritance of heterostyly in *Lythrum salicaria* **393**
Inheritance studies in soybeans, I Cotyledon color **441**

Inheritance studies in soybeans. II Glabrousness, color of pubescence, time of maturity and linkage relations **519**
INNES, W. T. *279*
Irish Cobbler 90
ISHIKAWA, C. *279*
ISHIWARA, MAKOTO 277, *279*
JEFFREY, E. C. 79, *82*
JENKINS, M. T. 416, 423, **492**, *504*
JENNINGS, H. S. 345, *347*
JOHANSSON, IVAR **93**
JONES, D. F. 487, *491*
JONES, E. 164, *165*
JORDON, D. S. *279*
KAJANUS, BIRGER 447, *447*
KANNA, BENZO 239, **242**
KAPLAN, D. M. 572, *580*
KAUFMANN, LAURA 550, 552, 553, *568*
KEITH, A. 572, *580*
KEMP, H. P. 284, *294*
KEMPTON, J. H. 115, *124*, *338*
KIRKHAM, W. B. 341, *347*
KNIGHT, 315
KOEHLER, B. *439*
KOPPÁNYI, T. 554, *568*
KOSSWIG, C. 531, *568*
KRAFKA, J. Jr. 50, *57*
KRANZ, P. 572, *580*
KVAKAN, PAUL, 437, *439*
LAIBACH, F. 395, *414*
Lebistes, 254, 276, 278
Lebistes reticulatus 266
Legitimate unions (Lythrum) 394
LENZ, F. 550, *568*
LEONHARDT, E. E. 256, *279*
Leontodon autumnaeis 65
LESLEY, M. M. **449**
LEWIS, DEAN *580*
LI, JU-CHI **1**
LIDFORRS, B. 336, *338*
LILLIE, R. S. 554, *568*
Limia couchia 256
vittata 254
LINDSTROM, E. W. 415, 416, 417, 424, *440*, 494, *504*, *529*
Linkage studies on mice **108**
Linum 395
LITARDIÉRE, de R. 81, *82*
LITTLE, C. C. 108, 109, 110, 111, 113, *114*, 164, *165*, 296, 297, 298, *306*, 344, 346, *347*
LJUNGDAHL, H. 69, 80, *82*
LOCK, 525, *568*
LOEB, J. 49, *57*
LOEB, LEO 554
LONGLEY, A. E. 73, 79, *82*, 336, 337, *339*
Lookout Mountain 90
LOWNE, B. T. 50, 51, *57*
LUCAS, H. C. 572, *580*
LUNERIA **447**
LUTMAN, 90
Lythrum salicaria 395, 396, 397, 408
Crosses between long styled and mid styled plants **400**
Crosses between long styled and short styled plants **401**
Crosses between mid styled and short styled plants **405**
Inheritance of heterostyly in **393**
Self fertilization 397
MACDOWELL 50
MCFARLANE, J. M. 258, *279*
MAGNUS, P. 60, *82*
Maize 115, 244, **348**
Algeria × Mexico Black 122
Chemical composition of pollen from sugary and non-sugary plants 476
Chlorophyll deficiencies, Genetic study **415**
Comparative weights of S_u W_x, s_u w_x, S_uW_x, and s_uw_x seeds **478**
Differential action of the sugary gene **348**
Effects on the waxy ratio of the composition of the pistilate parent with respect to the sugary gene **357**
Evidence from backcrosses that the sugary gene depresses the waxy ratio **364**
Factor for yellow-green chlorophyll color in maize and its linkage relations **492**
Factor V_6, V_7 and Y **427**
Four County **423**
Greenish and light virescent **423**
Inheritance of yellow-green chlorophyll color **493**
Light virescent (v_6v_6) **418**
Liguleless and rolled **425**
Linkage of Y_gy_g with S_hS_h and W_xw_x **500**, **501**
Linkage relations **427**
Linkage relations of Y_gy_g **494**
Method of calculating the percentage of crossing over in F_2 progenies **495**
Order of genes Y_g–C–S_h–W_x **502**
Pollen mixtures **469**
Proportions of waxy seeds in the upper and lower hælves of the ear **464**
Rate of growth of sugary and non-sugary sprouts and digestion of the endosperm reserves during seedling development **480**
Ratio of waxy grains in pollen from sugary and non-sugary plants segregating for waxy **463**
Ratios obtained on selfing waxy heterozygotes, homozygous for the sugary and non-sugary genes, respectively **352**
Relation between the factors W_1 and S_u **431**
Relation between scarred endosperm (S_c) and sectored plants **436**
Rolled **418**, **421**
Rolled and anther ear dwarf **427**
Scarred endosperm **419**
Scarred endosperm and sectorial plants **421**
Scutellum rot **438**
Silver King **423**
Sugary and rolled **426**
Sugary gene in maize as a modifier of the waxy ratio **461**
Sugary ratio when S_uS_u W_xW_x plants are used as staminate parents **372**
Virescent and W_1 **421**
Virescent and Y, C and R **423**
Waxy ratio, on long and short silk **466**

Waxy ratio in the sugary and non-sugary classes **365**
White-base-leaf **419, 420**
Yellow-green plants **492**
Yellowish virescent 417
Male gametophyte (see maize)
Malus 324
MANGELSDORF, A. J. **307**, 397
MANGELSDORF, P. C. 397, **487**, *491*
MANN, M. C. *460*
Material inheritance of cotyledon colors (soybeans) **446**
MATTHIOLA
F_2 hybrids **455**
Incana 449, 459
Mendelian inheritance of chromosome shape **449**
Parental races and their F_1 hybrids **450**
Snowflake 449, 451, 452, 458, 540
MATTOON, E. *165*
MEEK, S. E. 254, 256, 257, 258, 259, 261, *279*
Meiosis and mitosis in normal and gynodimorphic *Ranunculus acris* **63**
Melandrium 447
Melanophores, The inheritance of two kinds in Platypoecilus **253**
Mendelian inheritance of chromosome shape in Matthiola **449**
METZ 2
MEYER, K. *82*
Mice (see Mouse)
A_y gene **341**
Chromosome constitution of Gates' "non-disjunction" (*v–o*) **379**
Chromosomes of Japanese waltzing **381**
Chromosomes of normal males **380**
Chromosomes of *v-o* males **381**
First maturation division **383**
Linkage studies **108**
Relation of brown to black-eyed white **109**
Relation of brown to piebald **110**
Relation of dilution to black-eyed white **111**
Relation of dilution to piebald **113**
Second maturation division **387**
Spermatogonia **381**
The *W* gene **344**
MILLARDET, A. 307, 325, **329**, 330, 331, 332, 334, 335, 336, 338, *339*
MILTON BRADLEY CO. Color wheel 533
Minnow **253**
Mirabilis 244, 252, 447
Mitosis and meiosis in normal and gynodimorphic *Ranunculus acris* L. **63**
MIYAKI, K. 239
MIYAZAWA, BUNGO 199, 200, *241*
MOHR, O. L. 2, 3, *57*, *165*
MOL, DE W. E. 80, *83*
Mollienisia sphenops 254
MOORE, A. R. 552, *568*
MORGAN, L. V. 27, 295, *306*
MORGAN, T. H. 2, 9, 12, 35, 50, 54, *57*, *58*, 93, 101, 102, 105, 106, *107*, 129, *165*, 277, *279* 294, *294*, 296, *306*, 345, *347*, 554, *568*
Morning-glory
Complemental factors **203**
Contracta 205, 206, 207, 208, 211, 239
Genetic analysis of white margined flowers **199**
Nandina 205, 206, 207, 208, 211, 214, 216, 219, 240
Quantitative variations of the white margin **200**
"Rangiku" 214
Segregation of 3 factors producing white margined flowers **220**
Special factor varying the quantity of white margin **226**
MORSE, W. J. 441, 447, *447*, 519
Mouse (see Mice)
A case of non-disjunction **295**
Functional inter-relationship of certain genes in the development of **340**
MULLER, H. J. 2, 130, *165*
MYERS, G. S. 259, 265, *279*
NAGAI, I. 444, 447, *447*, 519, 520, 521, 526, 528, *529*
NAWASCHIN, M. 65, *83*
NAWASCHIN, S. G. 60, 65, 67, 78, *83*
NĚMEC, B. 60, 63, 84, *92*, 284, *294*
Nicotiana 408
Non-disjunction in the mouse **295**
NORTHROP, J. H. 48, 49, 51, 52, *57*, *58*
NOVAK, E. *580*
Oenothera 90, 295
gigas 90
lata rubricalyx 90
ONSLOW, H. 552, 554, 563, 564, *568*
ONSLOW M. W. 553, *568*
Ornithogalum 66
narbonense 66
oligophyllum 66
umbellatum 66
OSSWALD, H. 263, *279*
OVEREEM, VAN C. 80, *83*
OWEN, F. V. **441**, **519**
Oxalis 393, 396
Oxalis valdiviana 395
PAINTER, T. S. 303, **379**, 380, *392*
Papaver 69, 80
PARKER, S. L. 49, *58*
Parthenocarpy (Fragaria) **322**
Parthenogenesis (Fragaria) **322**
PEARL, R. 49, *58*
PEARSON, KARL 416, *440*
PENNYPACKER, J. Y. *294*
PENZIG, O. 60, *83*
PETZEL, W. 572, *580*
Pharbitis Nil 199
Phaseolus multiflorus 319
Phaseolus vulgaris, L. 115, **284**, 285, 319
Inheritance of modification **287**
Some attempts to modify the germ plasm **284**
PHILLIPS, J. C. 108, 109, 111, 113, *114*
Poecilia couchii 256
PICTET, A. 48, *58*
Pigeons
Archangels 94
Crosses between ordinary pigeons and fantails **99**
Original stock **99**

F_1 offspring **101**
F_2 generation **101**
Backcross (F_1×ordinary pigeon) **102**
Other crosses 105
Fantails 105
Homer 94, 105
Inheritance in, VI Number of tail feathers and uropygial gland **93**
Number of tail feathers in ordinary pigeons **94**
Swallows 94
Tumbler 94
Uropygial gland in ordinary pigeons **97**
PIPER, C. V. 308, 441, 447, *447*
PISUM, 447, 525
PITTS, A. T. 572, *580*
Platypoecilus
Backcrosses **273**
couchia 254, 256
couchianus 256
Crosses
Gold ♀ by Red ♂ **268**
Gold by Stipple **275**
Red ♀ by Gold ♂ **269**
Cultural methods **265**
dominiensis 254
Inheritance of two kinds of melanophores **253**
maculatus 254, 256, 277
maculatus immaculatus 265
mantalis 254
Native habitat and variability **256**
nelsoni 254
perugiae 254
quitzeoensis 254
Taxonomy **254**
tropicus 254
variatus 254
Varieties
Black **263**
Gold **265**, 267
Red **263**, 267
Spotted **262**
Stippled **261**, 267
White 276
Varieties and their color patterns **259**
Pleiotropic genes 158
PLOUGH, H. H. *58*
Podophyllum peltatum 81
Poecilia couchiana 256
sphenops 254
Poeciliidae 256
Poeciliinae 256, 277, 278
Poecilus maculatus 256
Potentilla nepalensis 311, 322, **324**, 325, 338
sterilis 324, 325
Primula auricula 394
hirsuta 395
hortensis 395
sinensis 395
veris 393, 394
POTTENGER, F. M. *580*
Prunus 324
PRIZBRAM, H. 553, *568*
Pteris tremula 81
Pulmanaria 395
Pyrus 324
Ranunculus acris **59**, **60**, 62, 77, 81, 82
"Asepalis," 68, 69
Cytological and morphological investigations of gynodimorphic and normal forms **59**
Gynodimorphism **60**
Individuality of the choromsomes **66**
Mitosis and meiosis in the normal and gynodimorphic **63**
"Open buds" 68, 69
Progeny of a gynodimorphic plant crossed with normal **68**
subspecies, *boreauanus* **80**
Ranunculus bulbosus 60
polyanthemus 60
repens 79
var. *typicus* 80
RAWLES 579
REED, H. D. 253
REGAN, C. T. 254, 256, *279*
RENYI, G. S. 554, *568*
Reverse genovariation in *Drosophila funebris* **125**
Riccia 266
RICHARDSON, C. W. 326, 327, 330, *339*
RIDGWAY, ROBERT *279*, 493, *504*
Ridgway's color chart 261
ROBERTS, E. 50, *58*
ROMASHOFF, D. D. 129, *165*
Rosa 336
ROSENBERG, O. 80, *83*
ROSTRUP 339
Rubus 73, 324
SACHS, B. W. 265, *279*
SAITO, S. 520, 526, 528, *529*
SAKAMURA, T. 64, *83*, 284, *294*
Salvinianatans 81
SATO, K. *568*
SAUNDERS, E. R. 450, 451, 456, 457, *460*
SAX, K. 86, 90, 91, *92*, *491*, *529*
SCHMIDT, H. 60, *83*
SCHMIDT, JOHN 254, *279*
SCHREITMÜLLER, W. 255, 259, 262, *279*
SCHULTZ, W. 550, 552, *568*
Senecio vulgaris 81
SEYSTER, E. W. 50, *58*
SHARP, J. G. *580*
SHAW 117
SHULL, G. H. 447, *448*
SIMONDS, J. P. *580*
SINNOTT, E. W. *294*
SIRKS, M. J. 122, *124*
SLYE, MAUD 572, *580*
SMITH, HUGH B. **84**
Snapdragon 244
Soja max 441, 519
Solanaceae 84
Solanum chacoense 85, 86, 91
demissum 85, 86, 91
fendleri 85, 86, 91
jamesii 86, 91
lycopersicum 84
nigrum 84
tuberosum 86
Chromosome counts in varieties of **84**
var. Early Ohio 87, 89

var. McCormick 87, 89, 91
var. McIntyre 87, 89, 91
var. Russet Rural 89
SOLMS-LAUBACH, H. 307, 335, *339*
Some attempts to modify the germ plasm of *Phaseolus vulgaris* **284**
SOROKIN, HELEN **59**, 63, 80, *83*
Soybean
Aksarben 446, 447
Black eyebrow **520** 524, 529
Black eyebrow × glabrous **520**
Cross between green and glabrous **526**
Distinction between yellow and green cotyledons **441**
Duplicate factors for yellow cotyledons 443
Guelph 442
Inheritance studies in, I. Cotyledon color **441**
Inheritance studies in, II. Glabrousness, color of pubescence, time of maturity and linkage relations **519**
Ito San **447**
Linkage between P_1 and R_1 **521**
Manchu 446, 447
Mandarin 446, 447
Maternal inheritance of cotyledon colors **446**
Medium green 442, 446, 447
Relation of glabrousness to grouth and vigor **520**
Single factor for time of maturity **524**
STANSCH, K. 256, 259, *279*
STEIGLEDER, EMIL, 341, *347*
STEWART, R. T. 520, 528, *529*
STOUT, A. B. 91, *92*
Strawberry (see Fragaria)
STROMAN, G. N. 415, 416, 437, *440*
STRONG, L. E. 296
STRUVE, O. 259, 265, *279*
STUDENT 363, 367, *378*, 465, 468, 472, 474, 480
Studies in the causes of dental anomalies **570**
Studies on genetics of Fragaria **307**
Studies on inheritance in pigeons VI, Number of tail feathers and uropygial gland **93**
Studies on phenotypic manifestations of hereditary factors. I. On the phenotypic manifestation of the genovariation radius incompletus in *Drosophila funebris* **128**
STURTEVANT, A. H. 4, 9, 12, 24, 35, *58*, *165*
Sugary gene in maize as a modifier of the waxy ratio **461**
TABUCHI, K. 239
TÄCKHOLM, G. *339*
TAKEZAKI, YOSHINORI 199, 221, 240, *241*
TAYLOR, W. R. 65, 66, *83*
TERAO, H. 441, 447, *448*
TERASAWA, YASUFUSA 244, *252*
THADANI, K. J. 572, *580*
Thalictrum minus 81
THOMAS, N. 295, *306*
TIERNEY, J. 572, *579*
TIMME, W. 572, *580*,
TIMOFÉEFF-RESSOVSKY, E. A. 163
TIMOFÉEFF-RESSOVSKY, N. W. **125**, **128**, *165*
TISCHLER, G. 65, 79, *83*, 409, *414*
Top-minnow **253**
TORNIER 265
TOYAMA, K. 277, *280*
Tripsacum 337
TROLLAND, L. T. 552, *568*
TSCHERMAK, E. VON 116, *124*, 451, 456, *460*, 457, 525
TSHETVERIKOFF, S. S. 128
TURRILL, W. B. 60, *83*
TYZZER, E. E. 297, *306*
UBISCH, G. VON 395, 396, *414*
UHLENBUTH, E. *580*
UPHOF, J. C. TH. 319, *339*
Uromyces appendiculatus 115
Uropygial gland of pigeons **93**
Urtica 447
Vallisneria 266
VELONOVSKY, J. 60, *83*
Veronica 408
Vicia faba 284
Viriginia strawberry (see *Fragaria virginiana*)
de VRIES, HUGO 244, *252*, 457, *460*
WACHTER, W. L. **108**, 110, *114*
WARD, W. G. 572, *580*
WELLENSIEK, S. J. 525, *529*
WENTZ, J. B. 520, 528, *529*
WHEELON, H. 572, *580*
WHITING, P. W. 410
WILLIAMS 315
WILSON, E. B. 388, *392*
WILSON, I. T. *165*
WINGARD, S. A. **115**
WINGE, O. 254, 266, 276, *280*
WINKLER, H. 84, *93*, 553
WINTON, D. de 395, *414*
Wood strawberry (see *Fragaria vesca*) *448*
WOODWORTH, C. M. *439*, 441, 444, 445, 447, *448*, 526, *529*
WRIGHT, S. 108, *114*, **530**, 531, 532, 533, 536, 538, 554, 563, 565, 566, *568*, *569*
Xiphophorini 256
YARNELL, S. H. 397
YASUI, K. 69, *83*
YULE 524
ZEA (see Maize)
ZELENY, C., *165*
Zoogoneticus quitzeoensis 254

C30